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(54) **Intermediate compound for the preparation of halichondrin analogs**

Zwischenprodukt zur Herstellung von Halichondrin Analoga

Intermédiaire pour la préparation d'analogues d'halichondrine

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(56) References cited:
EP-A- 0 572 109 **WO-A-93/17690**

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EP 2 277 873 B1

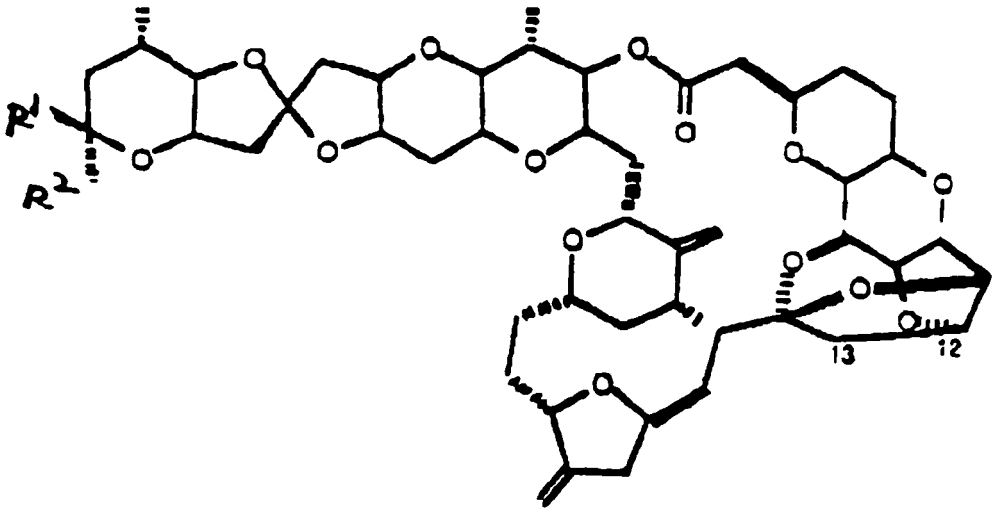
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Description

[0001] The invention relates to an intermediate for preparing pharmaceutically active macrolides. Halichondrin B is a potent anticancer agent originally isolated from the marine sponge *Halichondria okadai,* and subsequently found in *Axinella sp., Phakellia carteri,* and *Lissondendryx sp..*

[0002] A total synthesis of Halichondrin B was published in 1992 (Aicher, T.D. et al., J. Am. Chem. Soc. 114:3162-3164). Halichondrin B has demonstrated *in vitro* inhibition of tubulin polymerization, microtubule assembly, beta^{s}-tubulin crosslinking, GTP and vinblastine binding to tubulin, and tubulin-dependent GTP hydrolysis and has shown *in vitro* and *in vivo* anti-cancer properties.

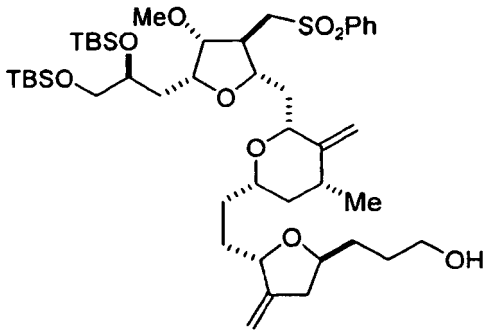
[0003] EP-A-0572109 discloses halichondrin derivatives of the formula:



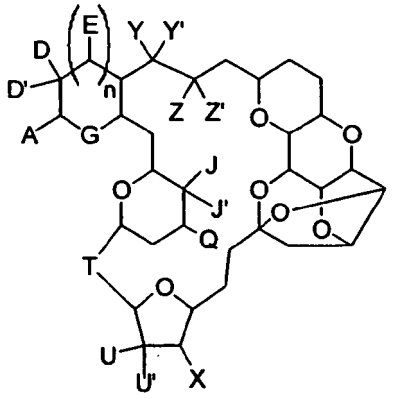
in which R^1 and R^2 together form certain bi- or tricyclic oxygen-containing fused ring systems substituted with a hydroxylated alkyl chain. These halichondrin derivatives have been found to have cytotoxic activity.

[0004] WO93/17690 discloses certain halichondrin derivatives that can be used to synthesise halichondrin B and norhalichondrin B. Some of these derivatives also exhibit anti-tumor activity.

The invention provides a compound having the formula:



The compound (ER804028) is useful as an intermediate for the preparation of halichondrin analogs having pharmaceutical activity, such as anticancer or antimitotic (mitosis-blocking) activity. These compounds are substantially smaller than halichondrin B and have the formula (I):



Formula (I)

[0005] In formula (I), A is a C_{1-6} saturated or C_{2-6} unsaturated hydrocarbon skeleton, the skeleton being unsubstituted or having between 1 and 13 substituents, preferably between 1 and 10 substituents, e.g., at least one substituent selected from cyano, halo, azido, Q_1, and oxo. Each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, NR_2R_1, $NR_2(CO)R_1$, $NR_2(CO)(CO)R_1$, $NR_4(CO)NR_2R_1$, $NR_2(CO)OR_1$, $(CO)OR_1$, $O(CO)R_1$, $(CO)NR_2R_1$, and $O(CO)NR_2R_1$. The number of substituents can be, for example, between 1 and 6, 1 and 8, 2 and 5, or 1 and 4. Throughout the disclosure, numerical ranges are understood to be inclusive.

[0006] Each of R_1, R_2, R_4, R_5, and R_6 is independently selected from H, C_{1-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} aminoalkyl, C_{6-10} aryl, C_{6-10} haloaryl (e.g., p-fluorophenyl or p-chlorophenyl), C_{6-10} hydroxyaryl, C_{1-4} alkoxy-C_6 aryl (e.g., p-methoxyphenyl, 3,4,5-trimethoxyphenyl, p-ethoxyphenyl, or 3,5-diethoxyphenyl), C_{6-10} aryl-C_{1-6} alkyl (e.g., benzyl or phenethyl), C_{1-6} alkyl-C_{6-10} aryl, C_{6-10} haloaryl-C_{1-6} alkyl, C_{1-6} alkyl-C_{6-10} haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heterocyclic radical-C_{1-6} alkyl, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl. There may be more than one R_1, for example, if A is substituted with two different alkoxy (OR_1) groups such as butoxy and 2-aminoethoxy.

[0007] Examples of A include 2,3-dihydroxypropyl, 2-hydroxyethyl, 3-hydroxy-4-perfluorobutyl, 2,4,5-trihydroxypentyl, 3-amino-2-hydroxypropyl, 1,2-dihydroxyethyl, 2,3-dihydroxy-4-perfluorobutyl, 3-cyano-2-hydroxypropyl, 2-amino-1-hydroxy ethyl, 3-azido-2-hydroxypropyl, 3,3-difluoro-2,4-dihydroxybutyl, 2,4-dihydroxybutyl, 2-hydroxy-2(p-fluorophenyl)-ethyl, $CH_2(CO)$(substituted or unsubstituted aryl), $-CH_2(CO)$(alkyl or substituted alkyl, such as haloalkyl or hydroxyalkyl) and 3,3-difluoro-2-hydroxypent-4-enyl.

[0008] Examples of Q_1 include -NH(CO)(CO)-(heterocyclic radical or heteroaryl), $-OSO_2$-(aryl or substituted aryl), -O(CO)NH-(aryl or substituted aryl), aminoalkyl, hydroxyalkyl, -NH(CO)(CO)-(aryl or substituted aryl), -NH(CO)(alkyl)(heteroaryl or heterocyclic radical), O(substituted or unsubstituted alkyl)(substituted or unsubstituted aryl), and -NH(CO)(alkyl)(aryl or substituted aryl).

[0009] Each of D and D' is independently selected from R_3 and OR_3, wherein R_3 is H, C_{1-3} alkyl, or C_{1-3} haloalkyl. Examples of D and D' are methoxy, methyl, ethoxy, and ethyl. In some embodiments, one of D and D' is H.

[0010] The value for n is 1 or preferably 0, thereby forming either a six-membered or five-membered ring. This ring can be unsubstituted or substituted, e.g., where E is R_5 or OR_5, and can be a heterocyclic radical or a cycloalkyl, e.g. where G is S, CH_2, NR_6, or preferably O.

[0011] Each of J and J' is independently H, C_{1-6} alkoxy, or C_{1-6} alkyl; or J and J' taken together are $=CH_2$ or -O-(straight or branched C_{1-5} alkylene or alkylidene)-O-, such as exocyclic methylidene, isopropylidene, methylene, or ethylene. Q is C_{1-3} alkyl, and is preferably methyl. T is ethylene or ethenylene, optionally substituted with $(CO)OR_7$, where R_7 is H or C_{1-6} alkyl. Each of U and U' is independently H, C_{1-6} alkoxy, or C_{1-6} alkyl; or U and U' taken together are $=CH_2$ or-O-(straight or branched C_{1-5} alkylene or alkylidene)-O-. X is H or C_{1-6} alkoxy. Each of Y and Y' is independently H or C_{1-6} alkoxy; or Y and Y' taken together are =O, $=CH_2$, or -O-(straight or branched C_{1-5} alkylene or alkylidene)-O-. Each of Z and Z' is independently H or C_{1-6} alkoxy; or Z and Z' taken together are =O, $=CH_2$, or -O-(straight or branched C_{1-5} alkylene or alkylidene)-O-.

A. Definitions
B. Halichondrin Analogs
C. Synthesis of Halichondrin Analogs

D. Pharmacological Activity
E. Uses

A. Definitions

[0012] The following terms are defined in part below and by their usage herein.

[0013] Hydrocarbon skeletons contain carbon and hydrogen atoms and may be linear, branched, or cyclic. Unsaturated hydrocarbons include one, two, three or more C-C double bonds (sp^2) or C-C triple bonds (sp). Examples of unsaturated hydrocarbon radicals include ethynyl, 2-propynyl, 1-propenyl, 2-butenyl, 1,3-butadienyl, 2-pentenyl, vinyl (ethenyl), allyl, and isopropenyl. Examples of bivalent unsaturated hydrocarbon radicals include alkenylenes and alkylidenes such as methylidyne, ethylidene, ethylidyne, vinylidene, and isopropylidene. In general, compounds of the invention have hydrocarbon skeletons ("A" in formula (I)) that are substituted, e.g., with hydroxy, amino, cyano, azido, heteroaryl, aryl, and other moieties described herein. Hydrocarbon skeletons may have two geminal hydrogen atoms replaced with oxo, a bivalent carbonyl oxygen atom (=O), or a ring-forming substituent, such as -O-(straight or branched alkylene or alkylidene)-O- to form an acetal or ketal.

[0014] C_{1-6} alkyl includes linear, branched, and cyclic hydrocarbons, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isopentyl, sec-pentyl, neo-pentyl, tert-pentyl, cyclopentyl, hexyl, isohexyl, sec-hexyl, cyclohexyl, 2-methylpentyl, tert-hexyl, 2,3-dimethylbutyl, 3,3-dimethylbutyl, 1,3-dimethylbutyl, and 2,3-dimethyl but-2-yl. Alkoxy (-OR), alkylthio (-SR), and other alkyl-derived moieties (substituted, unsaturated, or bivalent) are analogous to alkyl groups (R). Alkyl groups, and alkyl-derived groups such as the representative alkoxy, haloalkyl, hydroxyalkyl, alkenyl, alkylidene, and alkylene groups, can be C_{2-6}, C_{3-6}, C_{1-3}, or C_{2-4}.

[0015] Alkyls substituted with halo, hydroxy, amino, cyano, azido, and so on can have 1, 2, 3, 4, 5 or more substituents, which are independently selected (may or may not be the same) and may or may not be on the same carbon atom. For example, haloalkyls are alkyl groups with at least one substituent selected from fluoro, chloro, bromo, and iodo. Haloalkyls may have two or more halo substituents which may or may not be the same halogen and may or may not be on the same carbon atom. Examples include chloromethyl, periodomethyl, 3,3-dichloropropyl, 1,3-difluorobutyl, and 1-bromo-2-chloropropyl.

[0016] Heterocyclic radicals and heteroaryls include furyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathienyl, 2H-pyrrolyl, pyrrolyl, imidazolyl (e.g., 1-, 2- or 4- imidazolyl), pyrazolyl, isothiazolyl, isoxazolyl, pyridyl (e.g., 1-, 2-, or 3- pyridyl), pyrazinyl, pyrimidinyl, pyridazinyl, indolizinyl, isoindolyl, 3H-indolyl, indolyl (e.g., 1-, 2-, or 3-indolyl), indazolyl, purinyl, 4H-quinolizinyl, isoquinolyl, quinolyl, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, pyrrolinyl, pyrrolidinyl, imidazolidinyl, pyrazolidinyl, pyrazolinyl, piperidyl, piperazinyl, indolinyl, isoindolinyl, and morpholinyl. Heterocyclic radicals and heteroaryls may be linked to the rest of the molecule at any position along the ring. Heterocyclic radicals and heteroaryls can be C_{2-9}, or smaller, such as C_{3-6}, C_{2-5}, or C_{3-7}.

[0017] Aryl groups include phenyl, benzyl, naphthyl, tolyl, mesityl, xylyl, and cumenyl.

[0018] It is understood that "heterocyclic radical", "acryl", and "heteroaryl" include those having 1, 2, 3, 4, or more substituents independently selected from lower alkyl, lower alkoxy, amino, halo, cyano, nitro, azido, and hydroxyl. Heterocyclic radicals, heteroaryls, and aryls may also be bivalent substituents of hydrocarbon skeleton "A" in formula (I).

B. Halichondrin Analogs

[0019] Referring to formula (I) above, the halichondrin analogs include compounds wherein n is 0; wherein each of D and D' is independently selected from R_3, C_{1-3} alkoxy, and C_{1-3} haloalkyloxy; wherein R_5 is selected from H, C_{2-6} alkyl, C_{1-6} haloalkyl, C_{1-6} hydroxyalkyl, C_{1-6} aminoalkyl, C_{6-10} aryl, C_{6-10} haloaryl, C_{6-10} hydroxyaryl, C_{1-3} alkoxy-C_6 aryl, C_{6-10} aryl-C_{1-6} alkyl, $C_{1-6\,6}$ alkyl-C_{6-10} aryl, C_{6-10} haloaryl-C_{1-6} alkyl, C_{1-6} alkyl-C_{6-10} haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heterocyclic radical-C_{1-6} alkyl, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl; and combinations thereof.

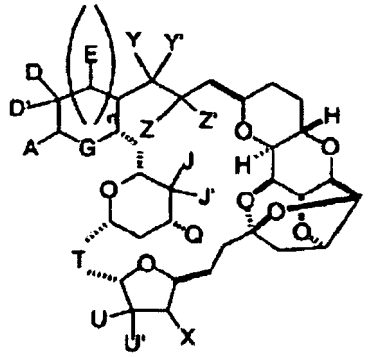
[0020] The analogs include compounds having one or more of the following characteristics: (a) wherein A is a C_{1-6} saturated or C_{2-6} unsaturated hydrocarbon skeleton, the skeleton having at least one substituent selected from cyano, halo, azido, Q_1, and oxo; (b) each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, NR_2R_1, $NR_2(CO)R_1$, and $O(CO)NR_2R_1$; (c) Z and Z' taken together are =O or $=CH_2$; (d) wherein each Q_1 is independently selected from OR_1, SR_1, SO_2R_1, OSO_2R_1, $NH(CO)R_1$, $NH(CO)(CO)R_1$, and $O(CO)NHR_1$; (e) each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy-C_6 aryl, C_6 aryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 aryl, C_6 haloaryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 haloaryl, (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl, C_{2-9} heterocyclic radical, C_{2-9} heteroaryl, and C_{2-9} heteroaryl-C_{1-6} alkyl; (f) one of D and D' is methyl or methoxy and the other is H; (g) n is 0; (h) G is O; (i) J and J' taken together are $=CH_2$; (j) Q is methyl; (k) T is ethylene; (l) U and U' taken together are $=CH_2$; (m) X is H; (n) each of Y and Y' is H; and (o) Z and Z' taken together are =O. Examples of combinations are the combination of (h)-(m), the combination of (a) and (b), the combination of (f) and (h), and the combination of (h) and where one of D and D' is methyl and the other

is H. Two particularly preferred compounds are B1793 and B1939.

[0021] The analogs also include compounds wherein Q_1 is independently selected from OR_1, SR_1, SO_2R_1, and OSO_2R_1; and each R_1 is independently selected from C_{1-6} alkyl, C_{1-6} haloalkyl, C_6 aryl, C_6 haloaryl, C_{1-3} alkoxy-C_6 aryl, C_6 aryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 aryl, C_6 haloaryl-C_{1-3} alkyl, C_{1-3} alkyl-C_6 haloaryl, and (C_{1-3} alkoxy-C_6 aryl)-C_{1-3} alkyl. The analogs also include compounds wherein: one of D and D' is alkyl or alkoxy, where n is 1; (f) as above, where n is 1; E is alkoxy, where n is 1; n is 0, where one of D and D' is hydroxy and the other is H; and (f) as above, where n is 1 and E is methyl.

[0022] The analogs also include compounds wherein: (1) A has at least one substituent selected from hydroxyl, amino, azido, halo, and oxo; (2) A is a saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl, amino, and azido (e.g., B1793, B1939, B2042, and 81922); (3) A has at least two substituents independently selected from hydroxyl, amino, and azido; (4) A has at least two substituents independently selected from hydroxyl and amino (e.g., B2042); (5) A has at least one hydroxyl substituent and at least one amino substituent (e.g., B1939); (6) A has at least two hydroxyl substituents (e.g., B1793); (7) A is a C_{2-4} hydrocarbon skeleton that is substituted (e.g., B2004, B2037, B1920); (8) A is a C_3 hydrocarbon skeleton that is substituted (e.g., B1793, B1920, B1984, B1988, B1939, B1940, B2014); (9) A has an (S)-hydroxyl alpha to the carbon atom linking A to the ring containing G (e.g., B1793, B1939 or B1920) or an (R)-hydroxyl (e.g. B2013, B2042); and (10) A is a C_{1-6} saturated hydrocarbon skeleton having at least one substituent selected from hydroxyl and cyano (e.g., B2013, B2037). By (S)-hydroxyl is meant the configuration of the carbon atom having the hydroxyl group is (S). The analogs also include compounds which have at least two substituents on the carbon atoms (1) alpha and gamma, (2) beta and gamma, or preferably (3) alpha and beta to the carbon atom linking A to the ring containing G. The alpha, beta, and gamma carbon atoms can have an (R) or (S) configuration.

[0023] Preferred analog compounds are those having the formula (1) -A, shown below, wherein the substituents are identical to those defined above.

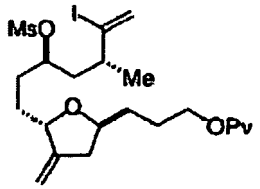


Formula 1-A

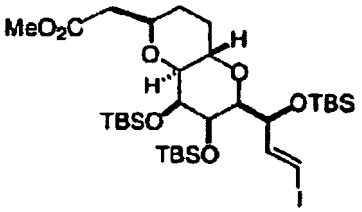
C. Synthesis of Halichondrin Analogs

[0024] An overview is provided below, followed by synthetic schemes 1-6, and several detailed protocols.

[0025] Compounds of general formula **4** can be prepared by the route outlined in Scheme 1. Key fragment **F-2** exemplified by vinyl iodide compound **X2** can be prepared according to the procedure of Kishi, et al (Total synthesis of halichondrin B and norhalichondrin B. Aicher, T. D.; Buszek, K. R.; Fang, F. G.; Forsyth, C. J.; Jung, S. H.; Kishi, Y.; Matelich, M. C.; Scola, P. M.; Spero, D. M.; Yoon, S. K. J. Am. Chem. Soc. 1992, 114, 3162-4).



Vinyl Iodide X2



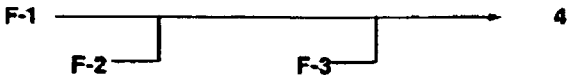
XF3

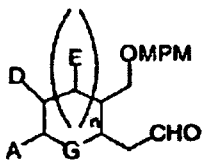
Key fragment **F-3** can be obtained by DIBALH reduction of the corresponding methyl ester, XF3, prepared according to the procedure of Stamos, et al (Scheme 2). [Synthetic studies on halichondrins: a practical synthesis of the C.1-C.13 segment. Stamos, D. P.; Kishi, Y. Tetrahedron Lett. 1996, 37, 8643-8646]. Synthesis of key fragment **F-1** exemplified by compound **20** can be synthesized as described in Scheme 3.

[0026] Ether analogs can be prepared from **B1793** by treatment with an appropriate alkylating agent (e.g. Scheme 4). Similarly, sulfonates, esters, carbamates, etc. can be prepared from **B1793** by treatment with an activated carbonyl component. Oxidative diol cleavage and reduction or selective hydroxyl group oxidation could furnish derivatives such as **B2037** and **B1934,** respectively.

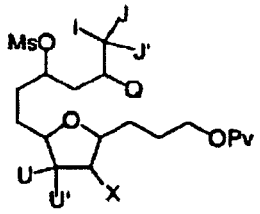
[0027] Alternatively, one or more hydroxyl groups could be converted to the corresponding amino groups with subsequent coupling with an activated carbonyl component (Scheme 5). Displacement of the sulfonyl intermediate (e.g. **B1920**) by carbon or heteroatom nucleophiles could also be readily accomplished (Scheme 6).

Scheme 1

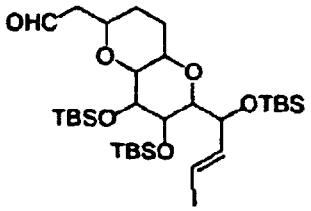




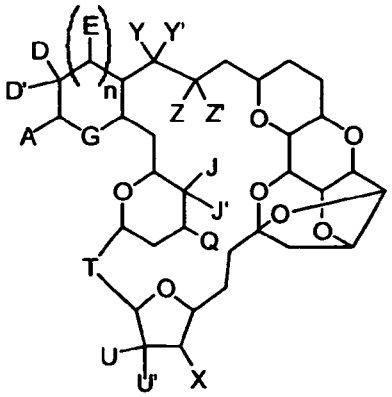
F-1



F-2

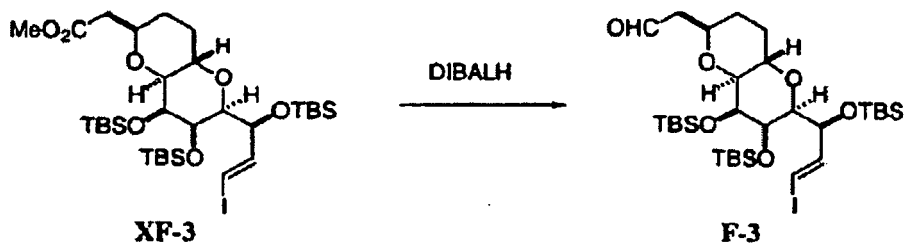


F-3



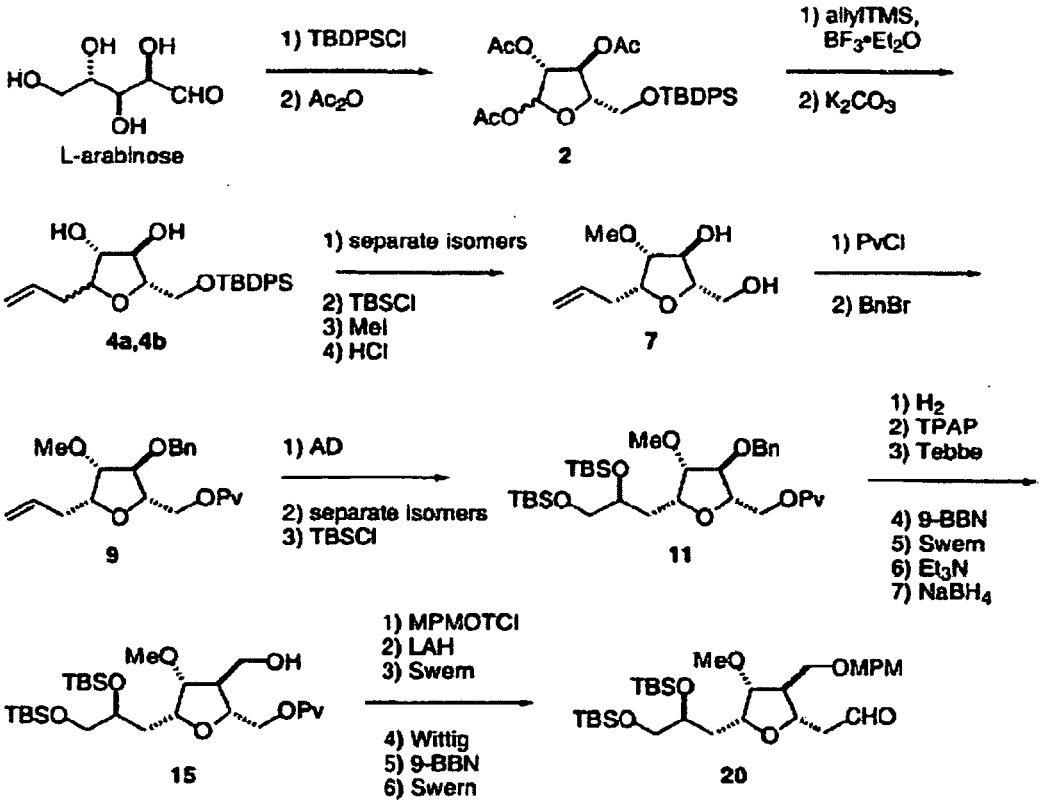
4, Formula (I)

Scheme 2

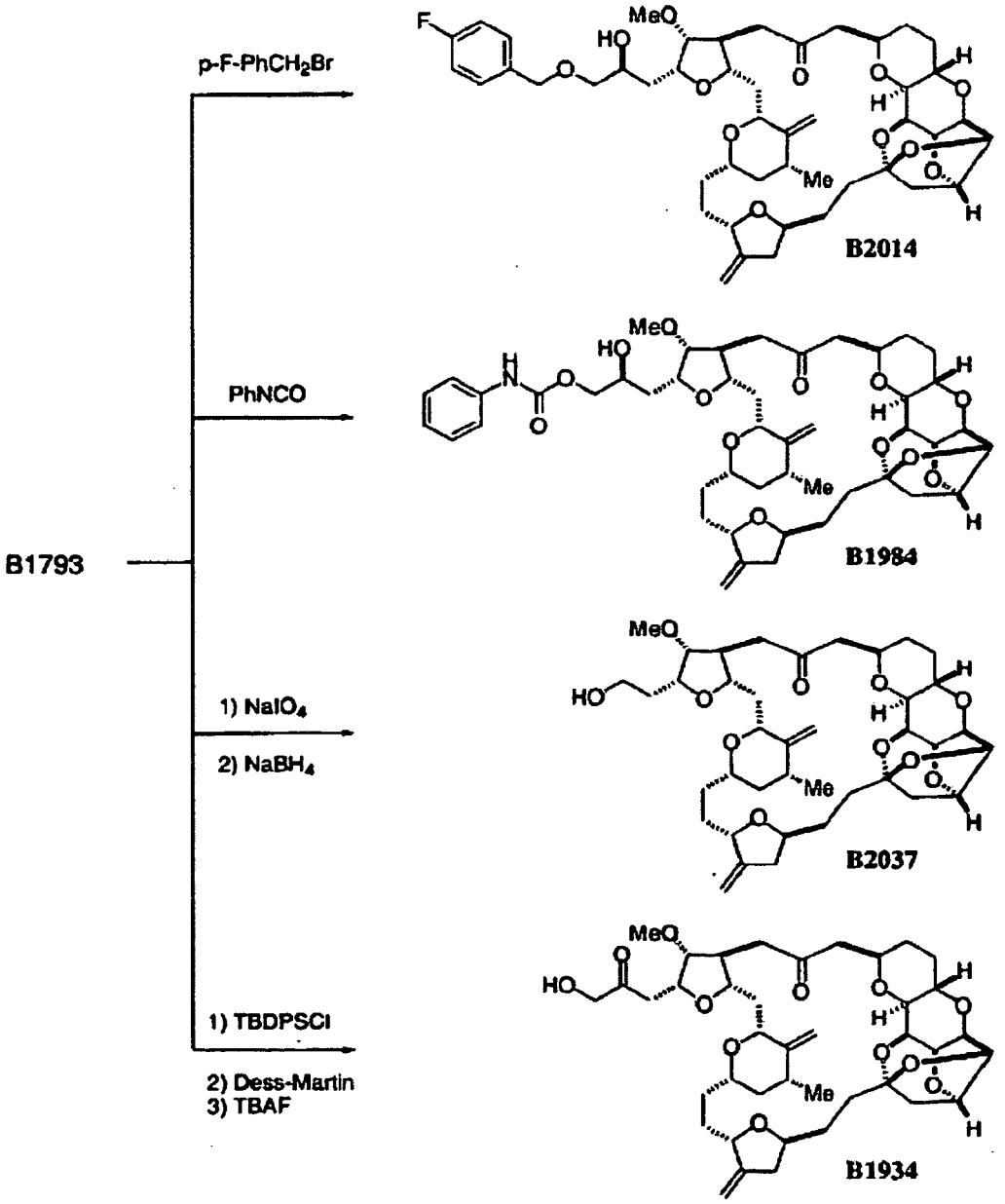


XF-3 F-3

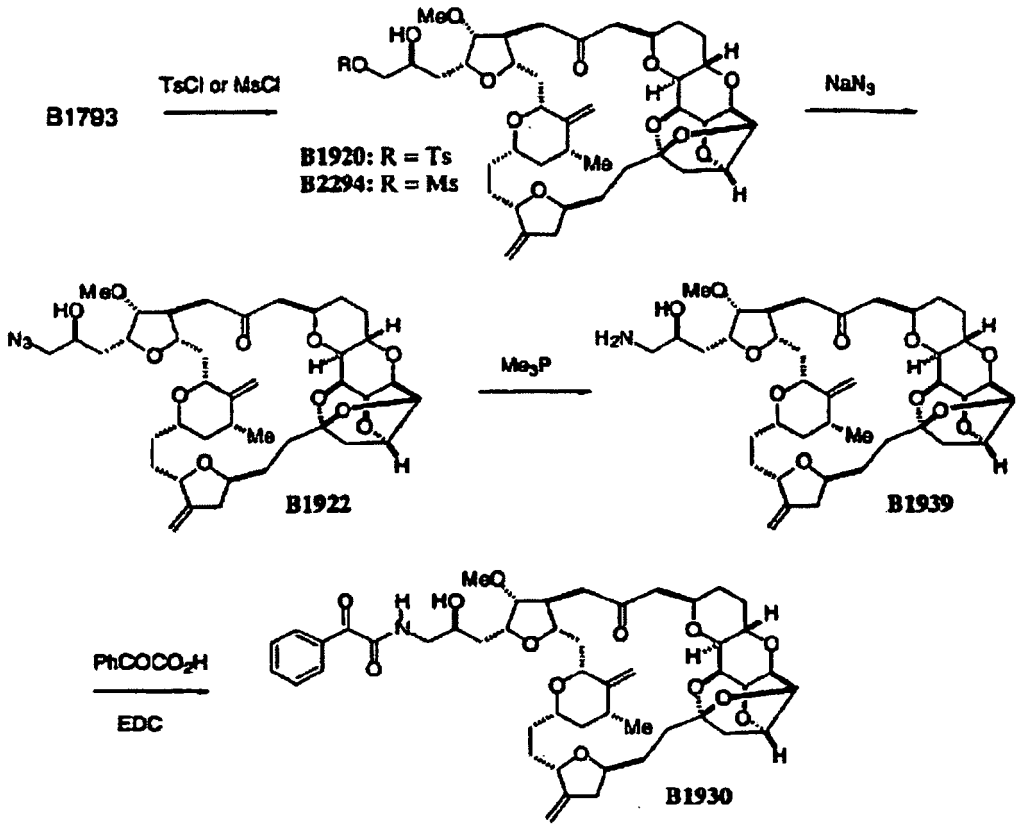
Scheme 3


L-arabinose
1) TBDPSCl
2) Ac2O
2
1) allylTMS, BF3•Et2O
2) K2CO3
4a,4b
1) separate isomers
2) TBSCl
3) MeI
4) HCl
7
1) PvCl
2) BnBr
9
1) AD
2) separate isomers
3) TBSCl
11
1) H2
2) TPAP
3) Tebbe
4) 9-BBN
5) Swern
6) Et3N
7) NaBH4
15
1) MPMOTCl
2) LAH
3) Swern
4) Wittig
5) 9-BBN
6) Swern
20

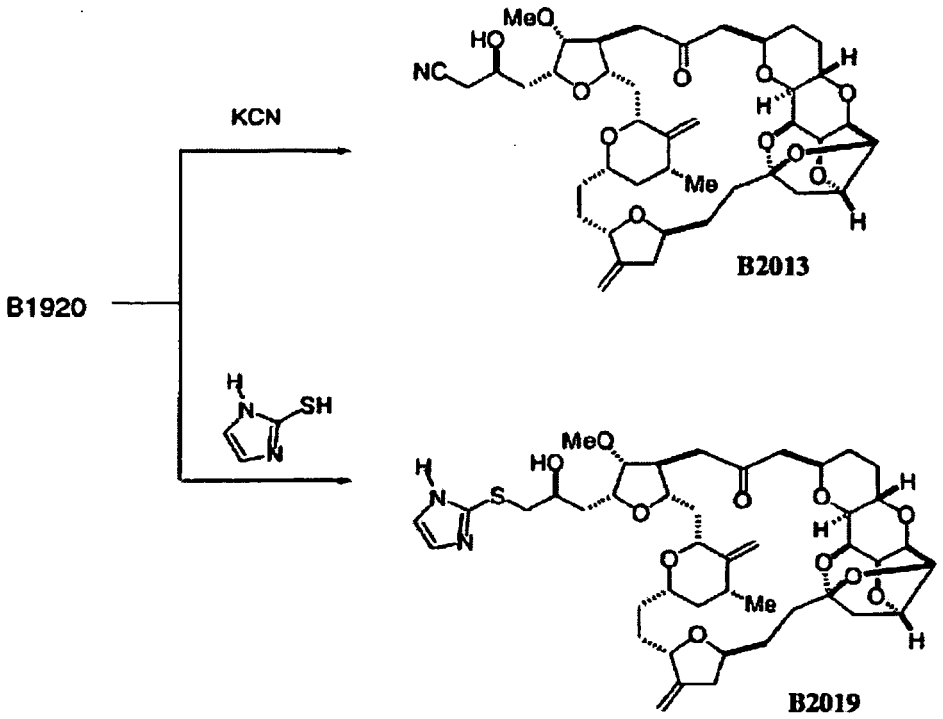
Scheme 4


B1793
p-F-PhCH2Br
PhNCO
1) NaIO4
2) NaBH4
1) TBDPSCl
2) Dess-Martin
3) TBAF
B2014
B1984
B2037
B1934

Scheme 5


B1793
TsCl or MsCl
B1920: R = Ts
B2294: R = Ms
NaN3
B1922
Me3P
B1939
PhCOCO2H
EDC
B1930

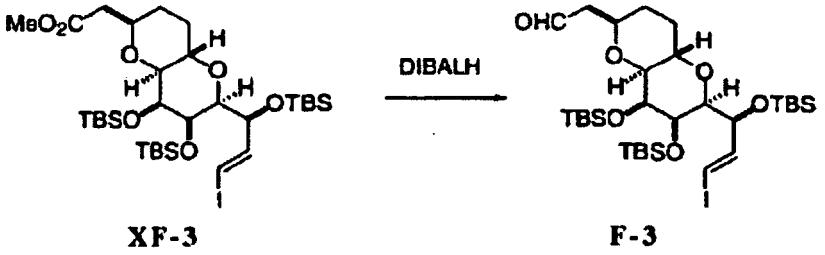
Scheme 6



EXPERIMENTAL SECTION

Synthesis of Key Fragment F-3:

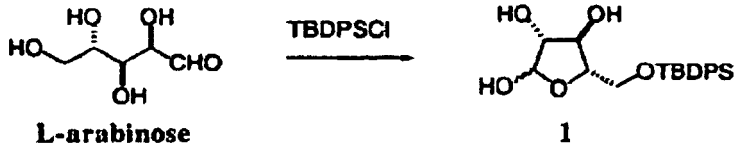
[0028]



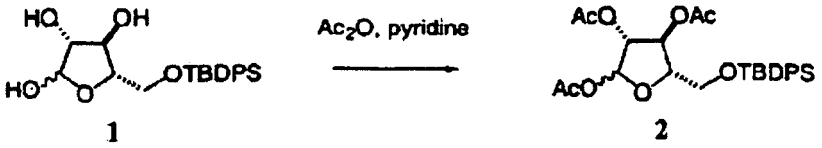
[0029] Key Fragment F-3. DIBALH (1 M in toluene, 3.86 mL) was added to a solution of **XF-3** (1,46 g, 1.93 mmol) in toluene (37 mL) at -78 °C. After stirring for 10 min, the reaction was quenched by careful addition of MeOH (0.46 mL) and H_2O (0.21 mL), warmed to rt and stirred for 15 min. The white suspension was filtered through Celite with 1:1 CH_2Cl_2/Et_2O. The filtrate was concentrated and purified by column chromatography (10% EtOAc-hexanes) to give key fragment F-3 (1.34 g, 96%) as an oil.

Synthesis of B1793:

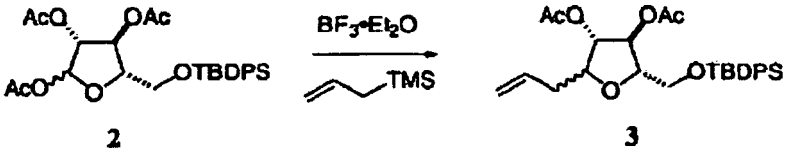
[0030]



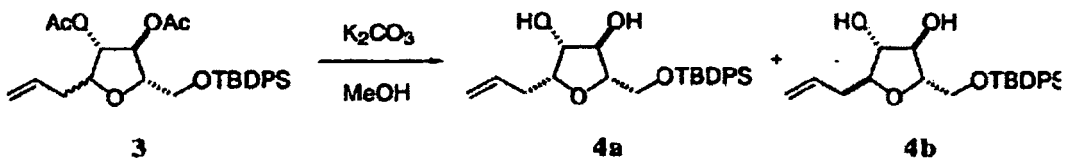
[0031] **Triol 1A** solution of TBDPSCl (444 mL, 1.7 mol) in DMF (0.5 L) was added in three portions to a suspension of L-arabinose (250.0 g, 1.66 mol), imidazole (231.4 g, 3.40 mol) and DMF (2.5 L). The addition of each portion took 1.5 h with a 30 min and a 15 h interval separating the second and third portions, respectively. The resulting solution was stirred for 3 h, concentrated and purified by flash chromatography (5% to 33% EtOAc-hexanes) to provide triol 1 (394 g, 61 %).



[0032] **Triacetate 2** Acetic anhydride (6.06 mol) was added over 1.5 h to triol 1 (1.01 mol) in pyridine (1.0 L) at 15 °C. The solution was stirred for 1 h, concentrated and purified by flash chromatography (15% to 25% EtOAc-hexanes) to afford triacetate 2 (518 g, 97%).

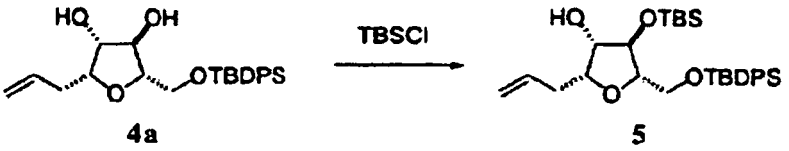


[0033] **Diacetates 3** Allyltrimethylsilane (1.11 mol) followed by $BF_3 \cdot OEt_2$ (1.11 mmol) was added over 1.5 h to triacetate **2** (164 g, 0.32 mol) in toluene (1.5 L) at 0 °C. The orange solution was stirred for 1 h at 0 °C and for 2 h at rt. The mixture was slowly poured into saturated aqueous $NaHCO_3$ (1.7 L) at 0 °C and stirred for 30 min. The separated aqueous layer was extracted with EtOAc (3 x 600 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% to 10% EtOAc-hexanes) to furnish a mixture of diacetates **3** (108 g, 69%).



[0034] **Diol 4a** Solid K_2CO_3 (72 mmol) was added to diacetates 3 (108 g, 218 mmol) in MeOH (0.5 L) at rt. The suspension was stirred for 2.5 h and then concentrated. The orange residue was suspended in saturated aqueous NH_4Cl (150 mL), extracted with EtOAc (3 x 150 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (15% to 50% EtOAc-hexanes) to afford alpha-isomer **4a** (33.86 g, 37%), and beta-

isomer **4b** (58 g, 63%).



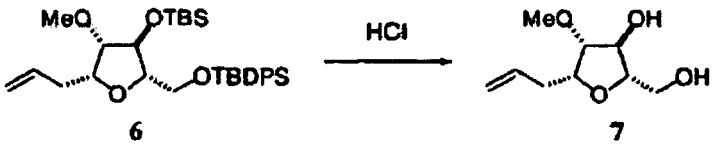
4a **5**

[0035] Alcohol 5 Imidazole (16.75 g, 246 mmol) and TBSCl (16.08 g, 107 mmol) were added to a solution of diol **4a** (33.86 g, 82 mmol) in CH_2Cl_2 (250 mL) at 0 °C. After 18 h at 0 °C and 5h at rt, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (250 mL), stirred for 30 min and the layers were allowed to separate. The aqueous layer was extracted with EtOAc (3 x 250 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (2% to 50% EtOAc-hexanes) to furnish alcohol **5** (36.0 g, 83%).



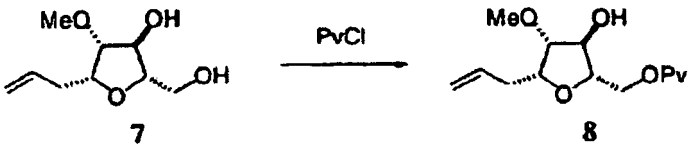
5 **6**

[0036] **Methyl ether 6** Iodomethane (16.5 mL, 265 mmol) and NaH (60% in mineral oil, 5.28 g, 132 mmol) were added to a solution of alcohol **5** (34.93 g, 66 mmol), THF (320 mL) and DMF (80 mL) at 0 °C. After 19 h at 0 °C, the reaction was quenched with saturated aqueous NH_4Cl and saturated aqueous $Na_2S_2O_3$. The resulting mixture was stirred for 20 min and the layers were allowed to separate. The aqueous phase was extracted with EtOAc (3 x 200 mL) and the combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (3% EtOAc-hexanes) to afford methyl ether **6** (34.23 g, 96%).



6 **7**

[0037] **Diol 7** HCl (37% aqueous solution, 12.75 mL, 153 mmol) was added to a solution of methyl ether **6** (32.93 g, 61 mmol) in MeOH (110 mL) at rt. After 17 h, $NaHCO_3$ (17 g) was added to the reaction mixture. The mixture was stirred for 30 min, concentrated, suspended in EtOAc and filtered. The filtrate was concentrated and purified by flash chromatography (50% EtOAc-hexanes to EtOAc) to give **diol 7** (10.0 g, 87%).



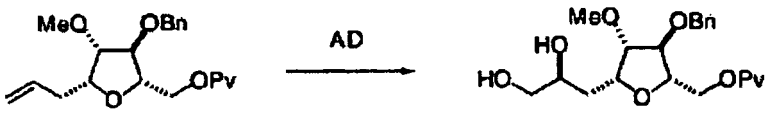
7 **8**

[0038] **Alcohol 8** A solution of pivaloyl chloride (8.4 mL, 67 mmol) in pyridine (50 mL) was added over 1.5 h to a solution of diol **7** (12.24 g, 65 mmol) in pyridine (100 mL) at 0 °C. After 1 h at 0 °C and 18 h at rt, the mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc (3 x 800 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (50% EtOAc-hexanes) to furnish alcohol **8** (16.9 g, 96%).



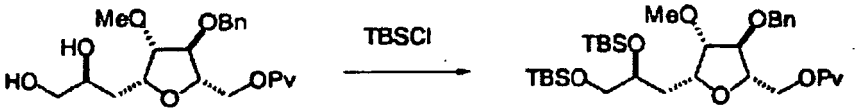
8 9

[0039] **Olefin 9** Benzyl bromide (62 mL, 521 mmol) and Bu_4NHSO_4 (10.6 g, 31 mmol) were added to a solution of alcohol **8** (16.9 g, 62 mmol) in CH_2Cl_2 (100 mL) at 0 °C. A solution of NaOH (9.95 g, 248 mmol) in H_2O (10 mL) was added to the reaction mixture over 15 min. After 30 min at 0 °C and 18 h at rt, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (hexanes to 30% EtOAc-hexanes) to afford olefin 9 (22.1 g, 98%).



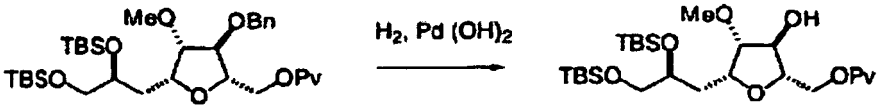
9 10a

[0040] **Diol 10a** OsO_4 (0.1M solution in toluene, 7.3 mL, 0.73 mmol) and a solution of olefin **9** (24.9 g, 69 mmol) in t-BuOH (165 mL) were added to a solution of K_2CO_3 (31.2 g, 161 mmol), $K_3Fe(CN)_6$ (74.4 g, 161 mmol), $(DHQ)_2PYR$ (1.33 g, 1.50 mmol), H_2O (500 mL) and t-BuOH (330 mL) at 0 °C. After 3 h at 0 °C, $Na_2S_2O_5$ 5 H_2O (37.3 g, 150 mmol) was added. The reaction mixture was warmed to rt, stirred for 1 h and extracted with EtOAc (3 x 300 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% isopropanol- CH_2Cl_2) to provide diol **10a** (17.98 g, 75%).



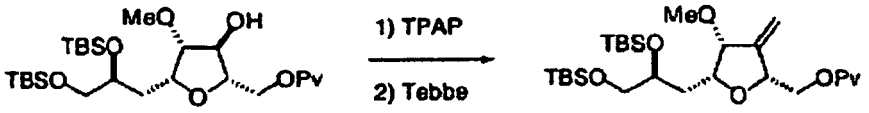
10a 11

[0041] **Silyl ether 11** Imidazole (21 g, 308 mmol) and TBSCl (26.5 g, 176 mmol) were added to a solution of diol **10a** (17.4 g, 44 mmol) in DMF (90 mL) at rt. After 18 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (250 mL), stirred for 1 h and extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were dried over Na_2SO_4, concentrated and purified by flash chromatography (5% EtOAc-hexanes) to afford silyl ether **11** (25.7 g, 94%).



11 12

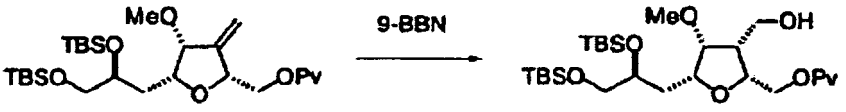
[0042] **Alcohol 12** A mixture of silyl ether **11** (21.2 g, 33.8 mmol), $Pd(OH)_2$ (20%, 4.7 g, 33.8 mmol) and EtOAc (200 mL) was stirred at rt under 1 atm H_2 for 3 h. The mixture was filtered through Celite, concentrated and purified by flash chromatography (10% to 20% EtOAc-hexanes) to afford alcohol **12** (17.4 g, 96%).



12 13

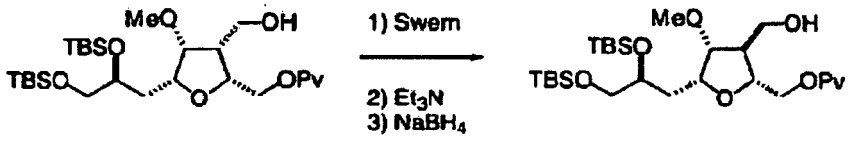
[0043] **Olefin 13** 4-Methylmorpholine N-oxide (7.66 g, 65 mmol) and TPAP (1.15 g, 3.26 mmol) was added in four portions over 20 min to a solution of alcohol **12** (17.4 g, 32.5 mmol) in CH_2Cl_2 (145 mL) at 0 °C. After 20 min, the reaction mixture was diluted with Et_2O (50 mL) and saturated aqueous $Na_2S_2O_5$ (50 mL) and filtered through Celite. The organic layer was separated, washed sequentially with saturated aqueous $CuSO_4$-brine (1:1) and brine, dried over Na_2SO_4, filtered through Celite, and concentrated to afford the desired crude ketone.

[0044] Tebbe reagent was prepared by stirring bis(cyclopentadienyl)titanium (11.36 g, 45.6 mmol) and Me_3Al (2.0 M in toluene, 45.6 mL, 91.2 mmol) for 4 days at rt. This material was cooled to -25 °C and a solution of crude ketone in THF (150 mL) was added. The reaction mixture was warmed to 0 °C, stirred for 30 min, quenched by slow addition of 0.1 N NaOH (3.5 mL), and then stirred for an additional 20 min at rt. The mixture was diluted with Et_2O, filtered through Celite and concentrated. The residue was dissolved in CH_2Cl_2, filtered through basic Al_2O_3, concentrated and purified by flash chromatography (5% EtOAc-hexanes) to give olefin **13** (12.8 g, 74% for two steps).



13 14

[0045] **Alcohol 14** 9-BBN (0.5 M in THF, 165 mL, 83 mmol) was added to a solution of olefin **13** (12.78 g, 24 mmol) in THF (280 mL) at 0 °C. After stirring for 5 h at rt, the reaction mixture was recooled to 0 °C at which time H_2O (200 mL), THF (100 mL) and $NaBO_3$•4 H_2O (75 g) were added. The mixture was warmed to rt, stirred for 16 h and then concentrated. The aqueous residue was extracted with EtOAc (4 x 300 mL) and the combined organic layers were dried over Na_2SO_4. Concentration and purification by flash chromatography (20% to 35% EtOAc-hexanes) afforded alcohol 14 (12.05 g, 91 %).



14 15

[0046] **Alcohol 15** DMSO (9 mL, 127 mmol) was added to a solution of oxalyl chloride (5.6 mL, 64 mmol) in CH_2Cl_{Z2} (350 mL) at -78 °C. After stirring for 15 min, a solution of alcohol **14** (11.7 g, 0.021 mmol) in CH_2Cl_2 (50 mL) was added and stirring was continued for 1 h, after which Et_3N (26.7 mL, 192 mmol) was added. The reaction mixture was warmed to 0 °C, stirred for 15 min, diluted with saturated aqueous NH_4Cl, and extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to furnish the desired crude aldehyde.

[0047] This material was dissolved in CH_2Cl_2 (200 mL) and treated with Et_3N (20 mL) at rt. After stirring overnight, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (3 x 200 mL). The combined organic layers were dried over Na_2SO_4, concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to afford the crude epimerized product.

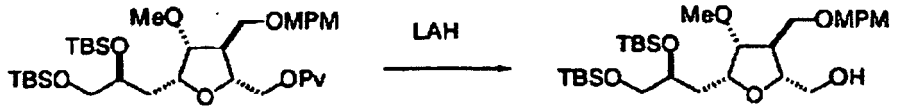
[0048] The aldehyde was dissolved in Et_2O-EtOH (1:1, 100 mL), cooled to 0 °C and treated with sodium borohydride (1.21 g, 32 mmol). The mixture was stirred for 20 min, carefully diluted with saturated aqueous NH_4Cl, stirred for 30 min at rt and extracted with CH_2Cl_2 (3 x 150 mL). The combined extracts were dried over Na_2SO_4, concentrated and purified

by flash chromatography (20% EtOAc-hexanes) to afford alcohol **15** (9.95 g, 85% for three steps).



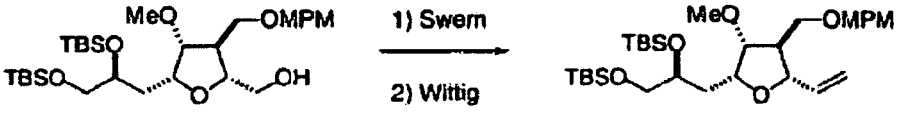
15 **16**

[0049] **MPM-ether 16** BF_3•OEt_2 (0.1 M in CH_2Cl_2, 1.8 mL, 0.18 mmol) was added to a solution of alcohol **15** (9.87 g, 18 mmol), MPM-trichloroimidate (4.9 mL, 27 mmol) and CH_2Cl_2 (175 mL) at 0 °C. After 40 min, a second portion of BF_3•OEt_2 (0.1 M in CH_2Cl_2, 0.9 mL, 0.09 mmol) was added to the reaction mixture. After 20 min, the reaction was quenched with saturated aqueous NH_4Cl, stirred for 1 h at rt and diluted with Et_2O (600 mL). The organic layer was separated and the aqueous layer was extracted with Et_2O (150 mL). The combined organic extracts were washed sequentially with 0.1 N aqueous NaOH, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to give MPM-ether **16** (10.20 g, 85%).



16 **17**

[0050] **Alcohol 17** LAH (1 M in THF, 22.5 mL, 22.5 mmol) was added to a solution of MPM-ether **16** (10.05 g, 15 mmol) in Et_2O (1.0 L) at 0 °C. After 30 min, the reaction was cautiously quenched with H_2O (1.3 mL), and 1 N aqueous NaOH (1.3 mL). After stirring for 1 h at rt, the suspension was filtered through Celite, concentrated and purified by flash chromatography (20% EtOAc-hexanes) to afford alcohol 17 (8.18 g, 93%).



17 **18**

[0051] **Olefin 18** DMSO (5.8 mL, 82.4 mmol) was added to a solution of oxalyl chloride (3.6 mL, 41.2 mmol) in CH_2Cl_2 (100 mL) at -78 °C. After 15 min, a solution of alcohol **17** (7.94 g, 13.5 mmol) in CH_2Cl_2 (35 mL) was added to the reaction mixture. After stirring for 1 h, Et_3N (17 mL, 122 mmol) was added, the mixture was warmed to 0 °C, stirred for 20 min, diluted with saturated aqueous NH_4Cl and then extracted with CH_2Cl_2 (3 x 100 mL). The combined organic extracts were dried over Na_2SO_4, concentrated and filtered through a short SiO_2 column (20% EtOAc-hexanes) to furnish the desired crude aldehyde.

[0052] *n*-BuLi (1.6 M, 20 mL, 30 mmol) was added dropwise to a solution of CH_3PPh_3Br (10.1 g, 30 mmol) in THF (350 mL) and DMSO (100 mL) at 0 °C. After 1 h, a solution of the crude aldehyde in THF (50 mL) was added. The reaction mixture was warmed to rt and stirred for 3 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with EtOAc (3 x 500 mL). The combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by flash chromatography (7% EtOAc-hexanes) to afford olefin 18 (5.57 g, 71% yield for 2 steps).



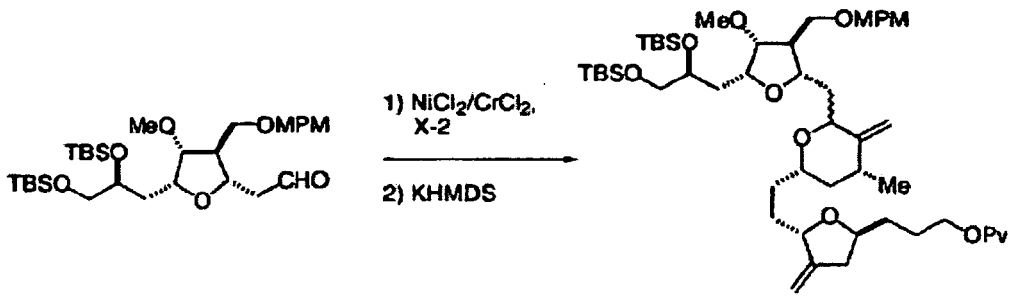
18 19

[0053] **Alcohol 19** 9-BBN (0.5 M in THF, 65 mL, 33 mmol) was added to a solution of olefin **18** (5.56 g, 9.6 mmol) in THF (85 mL) at 0 °C. The mixture was stirred for 5 h at rt and then recooled to 0 °C. H_2O (200 mL), THF (100 mL), and $NaBO_3$•4 H_2O (30 g) were sequentially added. After stirring overnight at rt, the organic volatiles were removed under reduced pressure. The aqueous residue was extracted with EtOAc (3 x 200 mL) and the combined organic layers were dried over Na_2SO_4. Concentration and purification by flash chromatography (30% EtOAc-hexanes) afforded alcohol **19** (12.05 g, 92%).



19 20

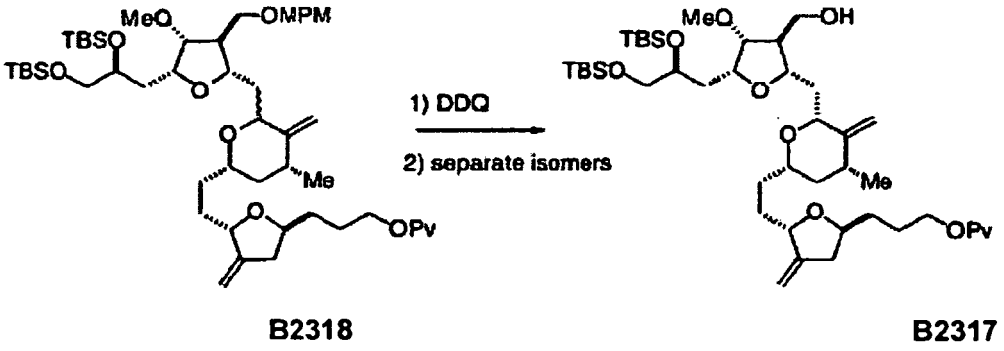
[0054] **Aldehyde 20**. DMSO (1.36 mL, 19.2 mmol) was added dropwise over 4 min to a solution of oxalyl chloride (1.26 mL, 14.4 mmol) in CH_2Cl_2 (120 mL) at -78 °C. After stirring for 10 min, a solution of alcohol **19** (5.76 g, 9.61 mmol) in CH_2Cl_2 (20 mL) was added via cannula. The transfer was completed by rinsing with additional CH_2Cl_2 (2 x 5 mL). After stirring for 20 min, the mixture was treated with Et_3N (5.36 mL, 38.4 mmol) and stirred for 10 min at -78 °C, 30 min at 0 °C and 10 min at rt. The reaction mixture was poured into saturated aqueous $NaHCO_3$ (200 mL) and the separated aqueous layer was extracted with CH_2Cl_2 (3x) followed by EtOAc (100 mL). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 20% EtOAc-hexanes) to furnish aldehyde compound **20** (5.28 g, 92%) as an oil.



20 B2318

[0055] **B2318**. 0.1% $NiCl_2/CrCl_2$ (w/w, 3.21 g) and 1% $NiCl_2/CrCl_2$ (w/w, 4.31 g) was added to a solution of aldehyde 20 (3.73 g, 6.25 mmol), key fragment **F-2** exemplified by vinyl iodide **X2.** (5.10 g, 9.16 mmol), THF (85 mL) and DMF (21 mL) at rt in a glove box. The reaction mixture was stirred for 24 h, removed from the glove box, cooled to 0 °C, diluted with EtOAc (100 mL), quenched with saturated NH_4Cl (200 mL) and stirred for 30 min. The separated aqueous phase was extracted with EtOAc (6x) and the combined organic layers were dried over Na_2SO_4 concentrated and purified by column chromatography (20% to 30%) to give **B2318** (~3 g) contaminated with close running impurities and the uncyclized intermediate (4.61 g). The latter (4.61 g, 4.48 mmol,) was dissolved in THF (150 mL), cooled to 0 °C and treated with KHMDS (0.5 M in toluene, 14 mL, 7.0 mmol) over a 2 min period. After stirring at 0 °C for 15 min, the reaction was quenched with saturated aqueous NH_4Cl (150 mL) and warmed to rt. The separated aqueous layer was extracted with EtOAc (3x) and the combined organic phases were dried over Na_2SO_4, concentrated and combined with the partially purified product obtained above. Column chromatography (10% EtOAc-hexanes) afforded **B2318** (3.17 g, 55%) as an

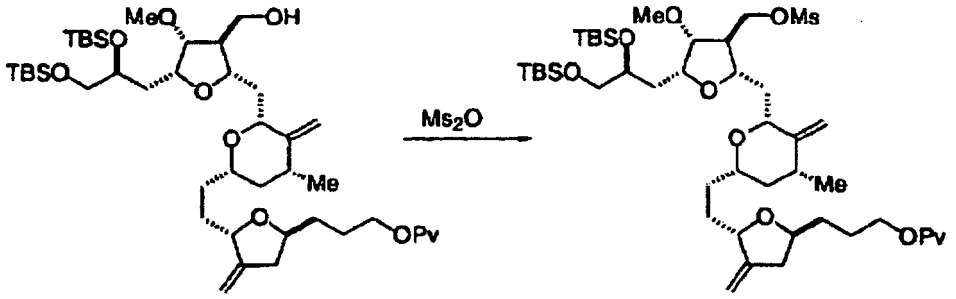
inseparable ~3:1 mixture of C27 diastereomers.



B2318 **B2317**

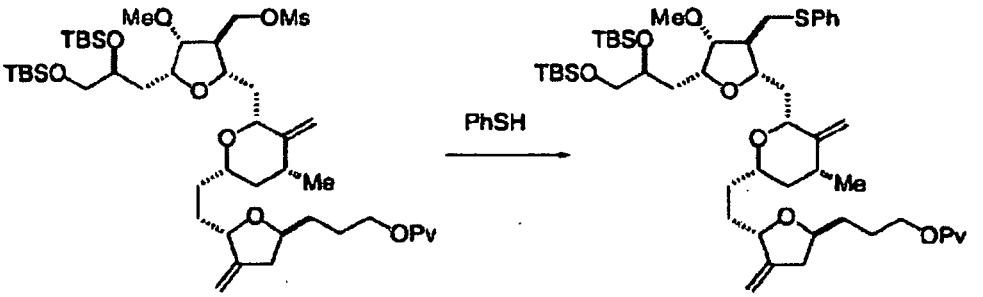
[0056] **B2317.** DDQ (1.45 g, 6.42 mmol) was added portion-wise over 30 min to a stirred solution of **B2318** (3.12 g, 3.35 mmol) in CH_2Cl_2 (50 mL) and pH 7 phosphate buffer (5 mL) at rt. The reaction was quenched with saturated aqueous $NaHCO_3$ (50 mL), stirred for 5 min, diluted with additional saturated aqueous $NaHCO_3$ (100 mL), H_2O (200 mL) and extracted with Et_2O (5x). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (15% to 30% EtOAc-hexanes) to give recovered **B2318** (1.40 g) and a mixture of the C27 isomeric products. The recovered **B2318** was resubmitted to the reaction conditions described above to afford additional product. Recovered starting material was again cycled through the deprotection conditions. All of the desired material was combined and separated by MPLC to afford **B2317** (1.65 g, 61%).

[0057] **B2304** may be prepared as follows.



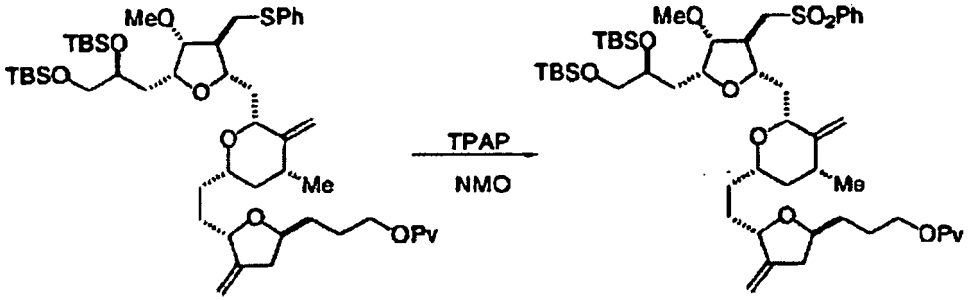
B2317 **ER804025**

[0058] To a solution of the alcohol, 2.4 g, in methylene chloride, 29 mL, was added methanesulfonyl anhydride, 770 mg. The mixture was stirred for 15 minutes, extracted with saturated sodium bicarbonate, dried and chromatographed to give 2.737 g, 100%.



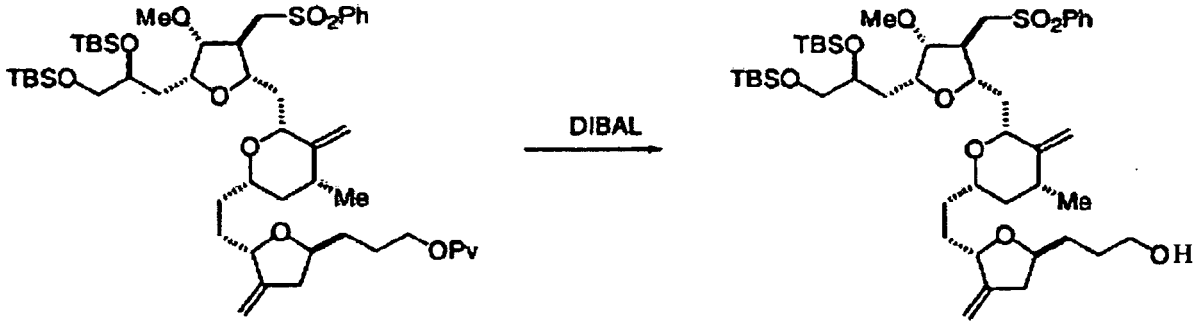
ER804025 ER804026

[0059] To a solution of the mesylate, 405 mg, in DMF, 0.06 mL, was added di-isopropylethylamine, 0.130 mL, followed by benzenethiol, 0.061 mL. After 4 hours and after 22 hours, additional amine, 0.03 mL, and benzenethiol, 0.015 mL, were added. After 24 hours, the mixture was diluted with 5% ethyl acetate/hexane. 1 mL and chromatographed to give 409 mg.



ER804026 ER804027

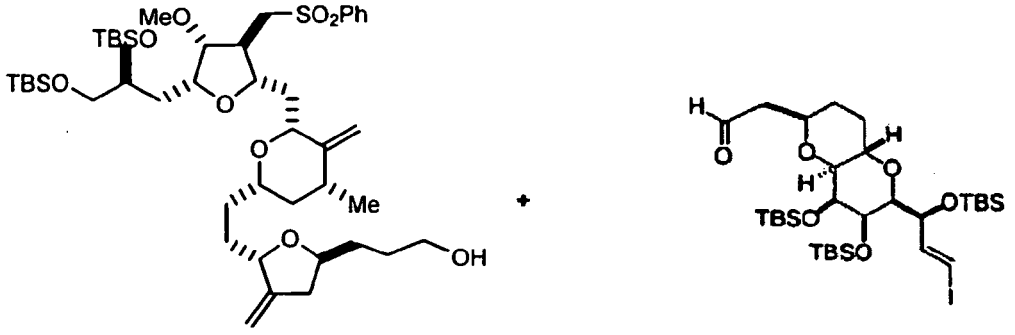
[0060] To a solution of the sulfide, 1.97 g, in acetonitrile, 16 mL, was added N-methylmorpholine oxide (NMO), and then a solution of 1.02 g, tetrapropylammonium perruthenate(VII), (TPAP), 38 mg, in acetonitrile, 1 mL. After 3.5 hours at room temperature, the mixture was heated to 40 °C for 1 hour. The mixture was cooled and aqueous satd. Sodium thiosulfate was added and the mixture partitioned between water and ethyl acetate. The usual work-up gave 1.567 g of a brown oil.



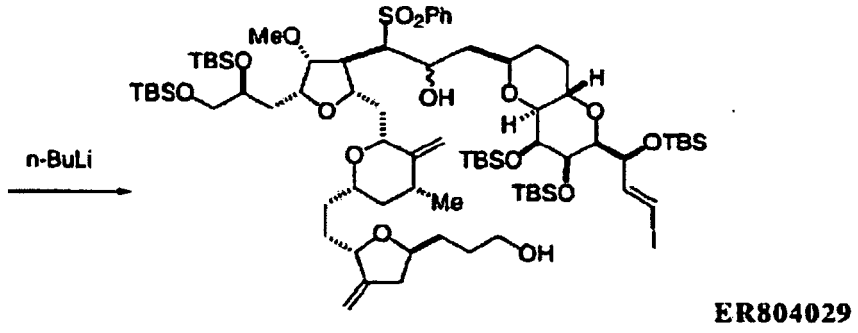
ER804027 ER804028

[0061] To a solution of the pivaloate ester, 1.567 g, in methylene chloride, 11.2 mL, at - 78 ° C in was added DIBAL,

2.5 mL of a 1 M solution in toluene. After 15 minutes, additional DIBAL, 0.8 mL, was added. After an additional 5 minutes, methanol, 0.46 mL, was slowly added followed by water, 0.2 mL. The mixture was filtered through Celite and chromatographed to give 1.386 g of an oil.

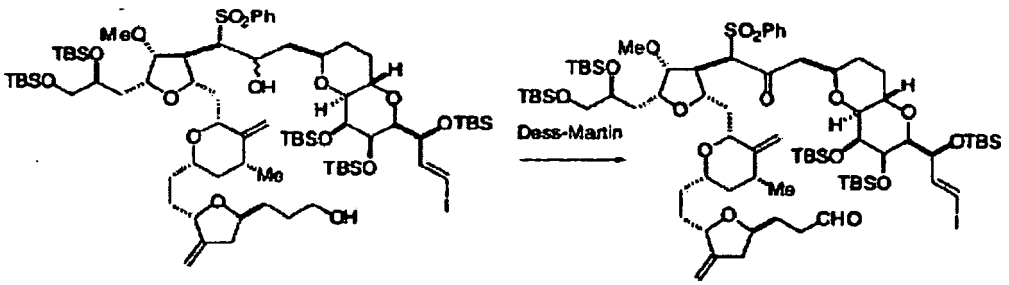


ER804028 F-3



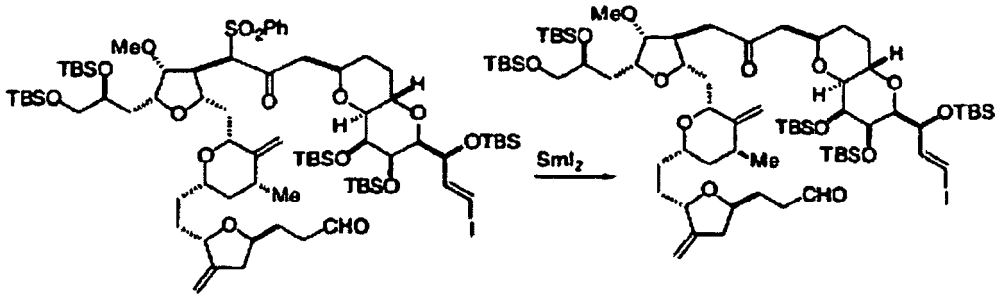
ER804029

[0062] To a solution of the sulfone, 36 mg, in DME, 1 mL, at -40 °C was added n-butyllithium, 2.8 equivalents. After 35 minutes, a solution of the aldehyde, 42 mg, in DME, 0.5 mL) was added. After 40 minutes, saturated aqueous ammonium chloride was added and the mixture extracted with ethyl acetate. The usual work-up, followed by chromatography gave 52 mg of an oil.



ER804029 ER804030

[0063] To a solution of the alcohol, 42 mg, in methylene chloride, 2 mL, was added the Dess Martin reagent, 36.4 mg. The mixture was stirred for 30 minutes and ether was added. The mixture was filtered through Celite, washed with saturated sodium bicarbonate, with saturated sodium thiosulfate, worked up in the usual way and chromatographed to give 38 mg of an oil.

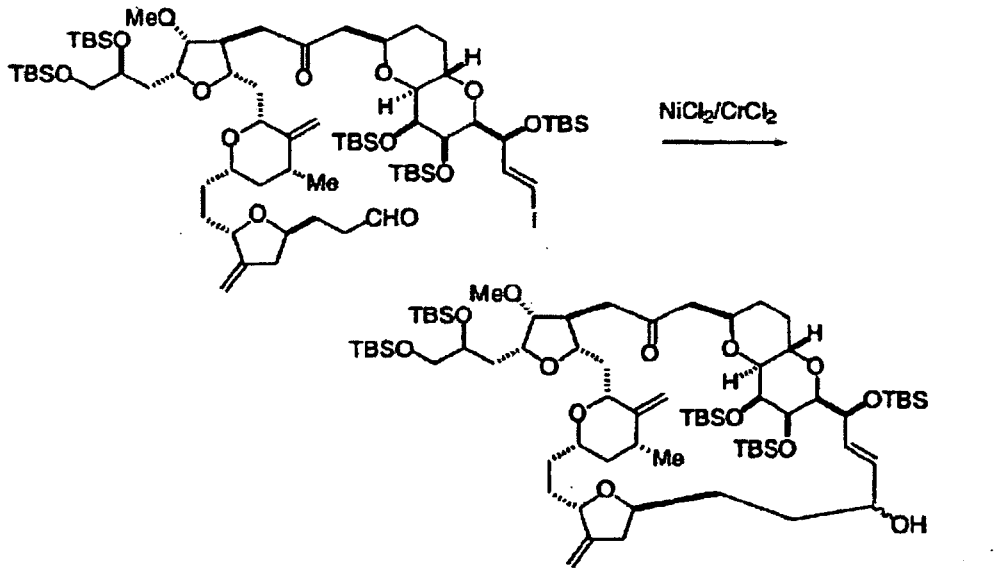


ER804030 **B2304**

Preparation of SmI_2 Solution

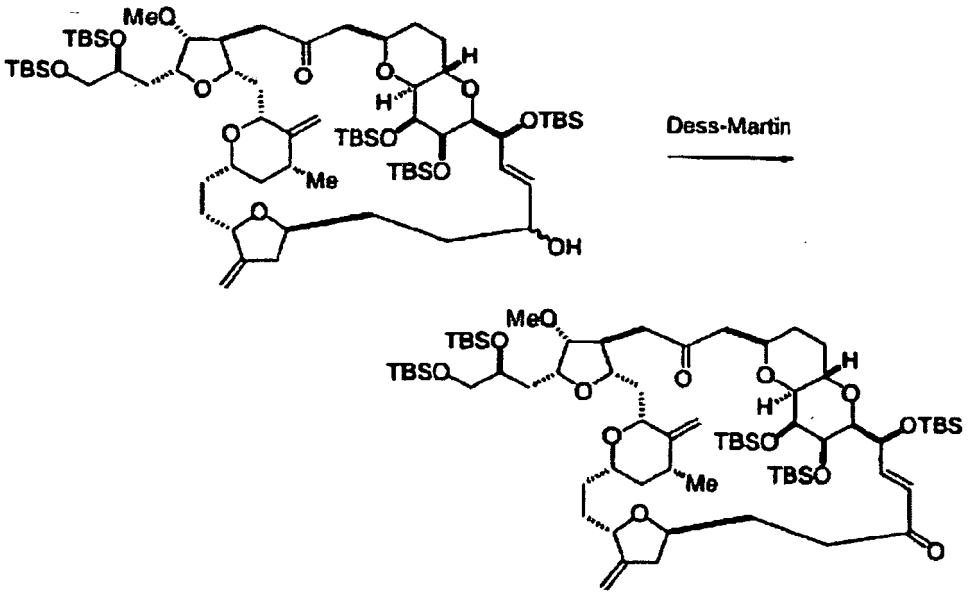
[0064] A solution of 1,2-di-iodoethane in 10 mL of THF was added to a suspension of Sm, 0.16 g, in THF, 1 mL. The mixture was stirred for 1 hour.

[0065] An aliquot of this solution, 0.03 mL, was added to a solution of the sulfone in THF at -78 ° C. After 5 minutes, additional SmI_2 reagent, 0.05 mL, was added. After a few additional minutes, more reagent, 0.25 mL, was added. The cooling bath was removed and saturated aqueous sodium bicarbonate, 3 mL, was added. The mixture was partitioned between ether and water and the usual work-up gave 9.1 mg, 81%, of an oil.



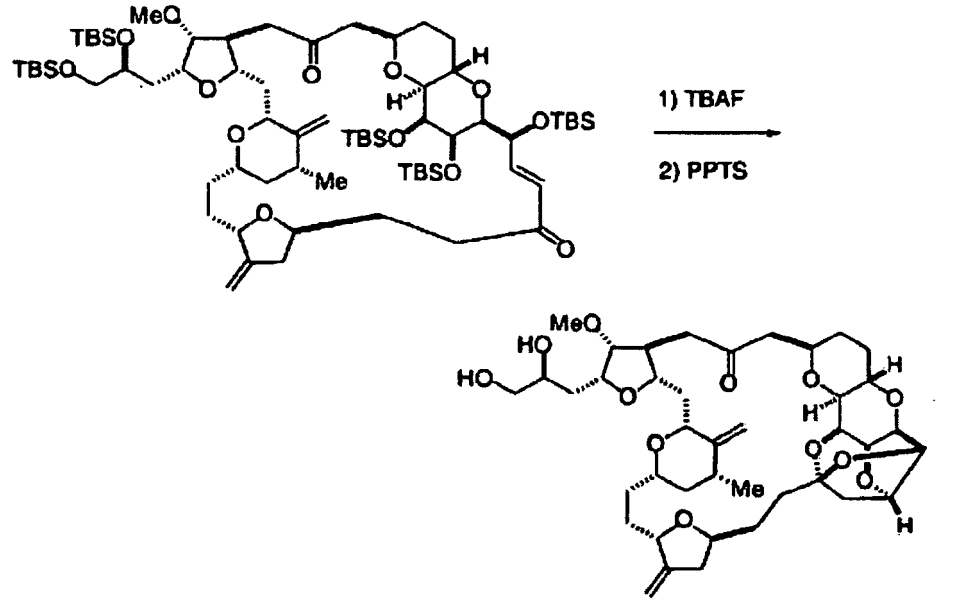
B2304 **B2302, B2303**

[0066] **B2302 and B2303.** In a glove box, $NiCl_2/CrCl_2$ (1% w/w, 1.09 g, 8.86 mmol) was added to a solution of **B2304** (1.01 g, 0.70 mmol) in THF (600 mL) and DMF (150 mL) at rt. After stirring for 2 days the reaction mixture was taken out of the glove box, cooled to 0 °C, quenched with saturated aqueous NH_4Cl (300 mL) and stirred at 0 °C for 20 min. After addition of H_2O (100 mL), the two layers were separated and the aqueous layer was extracted with EtOAc (5x). The combined organic phases were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (15% EtOAc-hexanes) to furnish a mixture of B2302 and B2303 (0.84 g, 92%) as a solid foam. Although the isomers could be separated by prep TLC (20% EtOAc-hexanes), they were carried forward as a mixture.



B2302, B2303 **B2301**

[0067] B2301. A mixture of **B2302/B2303** (0.79 g, 0.60 mmol) and Dess-Martin periodinane (0.26 g, 0.60 mmol) in CH_2Cl_2 (30 mL) at rt was stirred for 30 min. Additional Dess-Martin periodinane (0.26 g, 0.60 mmol) was added to the mixture and stirring was continued for additional 1.5 h. The mixture was then diluted with Et_2O (100 mL), stirred for 15 min and filtered through Celite. The filtrate was washed with saturated aqueous $NaHCO_3$ (100 mL) and the separated aqueous layer was extracted with Et_2O (3x). The combined organic phases were dried over Na_2SO_4, concentrated and purified by column chromatography (10% to 15% EtOAc-hexanes) to give **B2301** (0.67 g, 85%) as an oil.



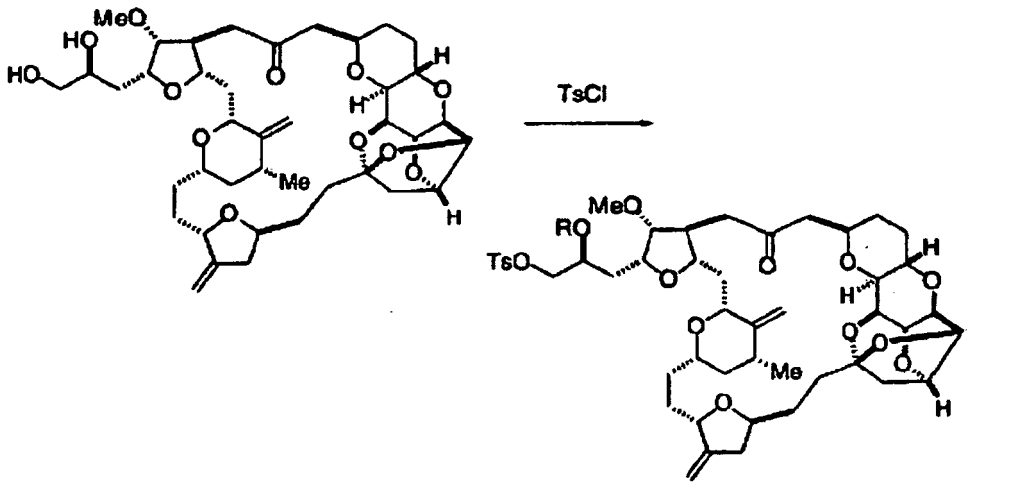
B2301 **B1793**

[0068] B1793. TBAF (1 M in THF containing 0.5 M imidazole HCl, 4.60 mL, 4.60 mmol) was added over 2 min to a solution of **B2301** (0.62 g, 0.48 mmol,) in THF (29 mL) at rt and the resulting mixture was stirred for 18 h. After dilution with hexanes (10 mL), the reaction mixture was directly loaded onto a SiO_2 column packed with 50% EtOAc-hexanes

and eluted with 50% EtOAc-hexanes (1 L) followed by 10% MeOH/EtOAc to collect a mixture of intermediates. After solvent removal, the residue was dissolved in CH_2Cl_2 (15 mL) and treated with PPTS (645 mg). After stirring for 1 h at rt, additional PPTS (414 mg) was added and the resulting white suspension was stirred for 4.5 h. The reaction mixture was then directly loaded onto a SiO_2 column packed with 70% EtOAc-hexanes and eluted with 70% EtOAc/hexanes (0.5 L), EtOAc (1 L). Elution with 5% to 10% MeOH/EtOAc furnished pure **B1793** (181 mg) and elution with 15% MeOH-EtOAc gave additional semi-pure product, which after purification by preparative TLC (10% MeOH-EtOAc) provided additional pure **B1793** (42 mg). **B1793** (total 223 mg, 64%) was obtained as a white solid. HRMS: calcd for $C_{40}H_{58}O_{12}$ + Na 753.3826. Found: 753.3808.

Synthesis of Representative B1793 Analogs:

[0069]

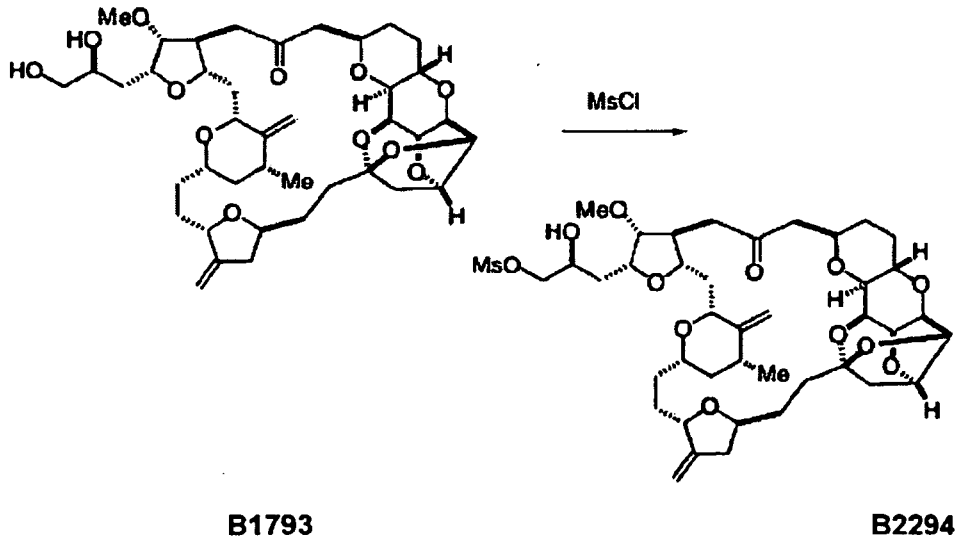


B1793

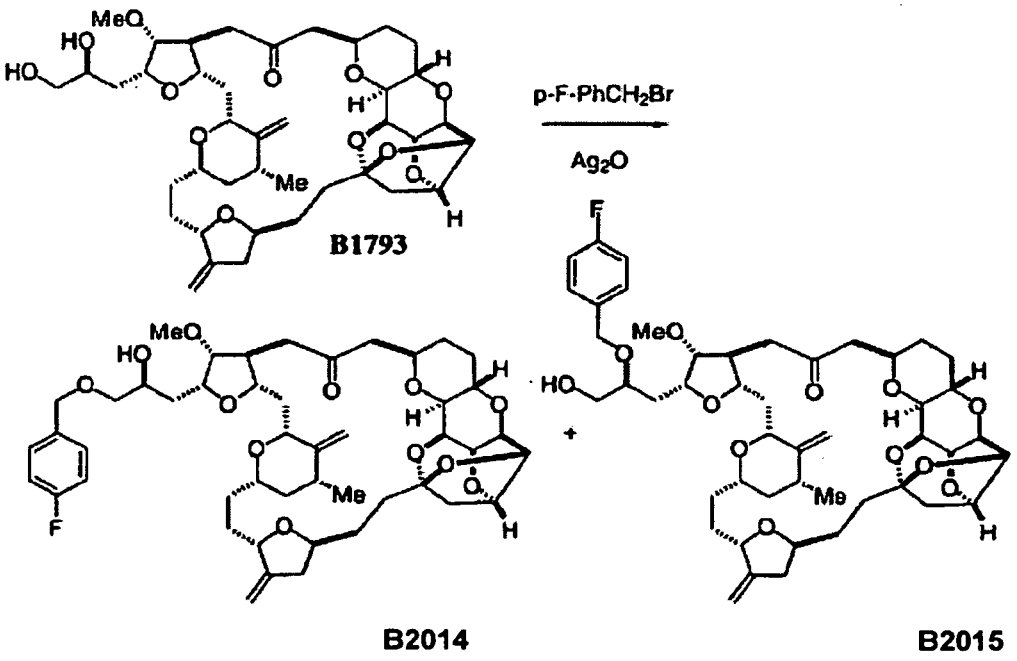
B1920: R = H

B1921: R = Ts

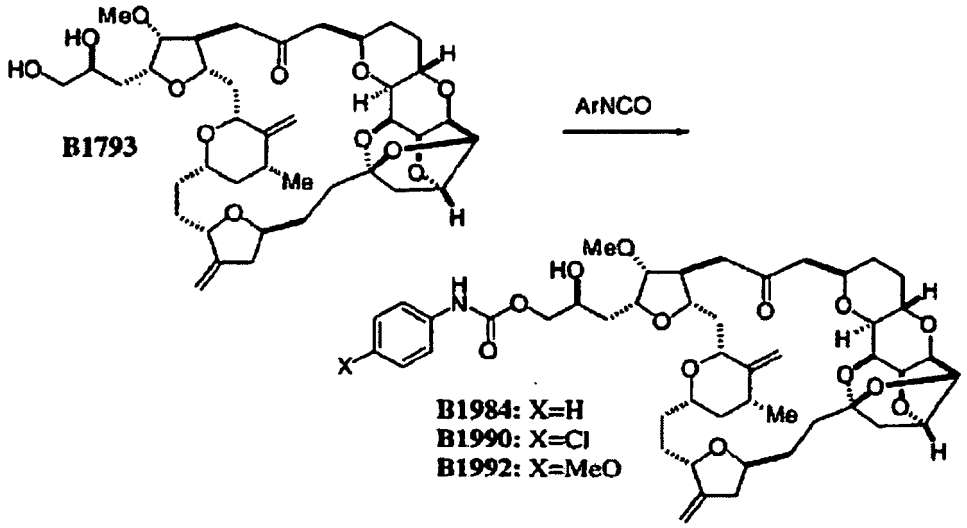
[0070] B1920 and B1921 TsCl (9.9 mg, 0.052 mmol) was added to a solution of diol **B1793** (7.6 mg, 0.010 mmol) in CH_2Cl_2 (1 mL) and pyridine (0.1 mL) at rt. After 48 h, the reaction was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (4x). The combined extracts were dried over Na_2SO_4 and concentrated. Purification by preparative TLC (80% EtOAc-hexanes) afforded monotosylate **B1920** (6.0 mg, 67%), and ditosylate **B1921** (1.8 mg, 18%).



[0071] **B2294** MsCl (0.3 M in CH_2Cl_2, 98 μL, 0.030 mmol) was added dropwise over 40 min to a mixture of collidine (7 μL, 0.054 mmol), **B1793** (20.8 mg, 0.028 mmol) and CH_2Cl_2 (1 mL) at 0 °C. After 76 h at 4 °C, the reaction was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (4x). The combined extracts were dried over Na_2SO_4 and concentrated. The crude product was dissolved in toluene (3 mL) concentrated and purified by preparative TLC (1.5% MeOH-EtOAc) to afford mesylate **B2294** (21.4 mg, 95%).

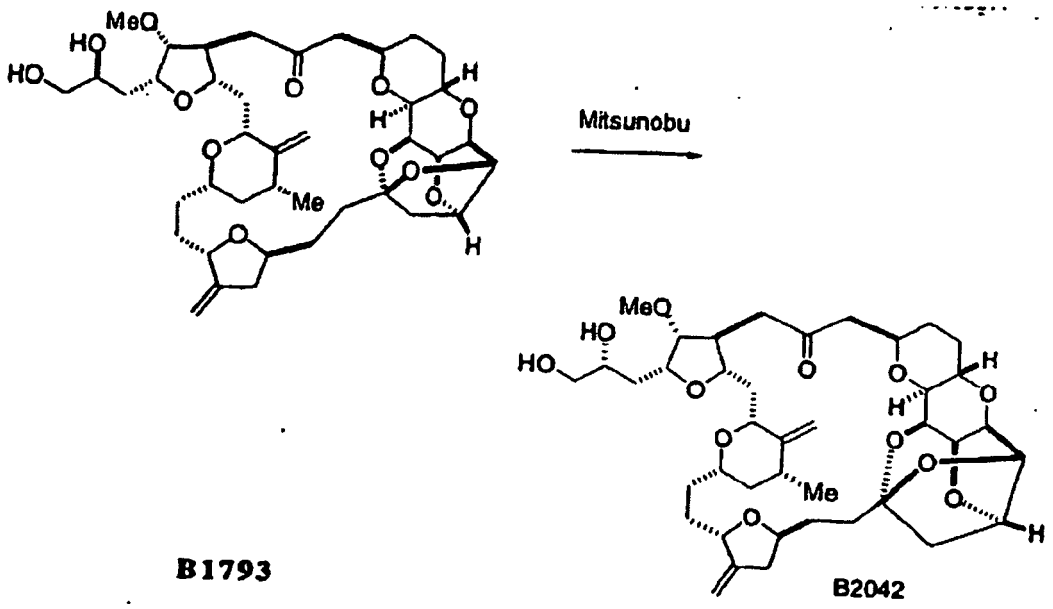


[0072] **B2014 and B2015** A 0.016 M solution of 4-fluorobenzyl bromide in Et_2O (800 μL, 13 μmol) and Ag_2O (10 mg, 43 μmol) were each added in three portions at 1 h intervals to a rt solution of **B1793** (1.7 mg, 2.3 μmol) in Et_2O (1.2 mL). The mixture was protected from light, stirred for 7 h and then filtered through Celite. Concentration and purification by preparative TLC (EtOAc) afforded primary ether **B2014** (1.1 mg, 56%), and secondary ether **B2015** (0.6 mg, 31%). HRMS (FAB): calcd for $C_{47}H_{63}FO_{12}$ + Na 861.4201. Found: for **B2014** 861.4178, for **B2015** 861.4160.

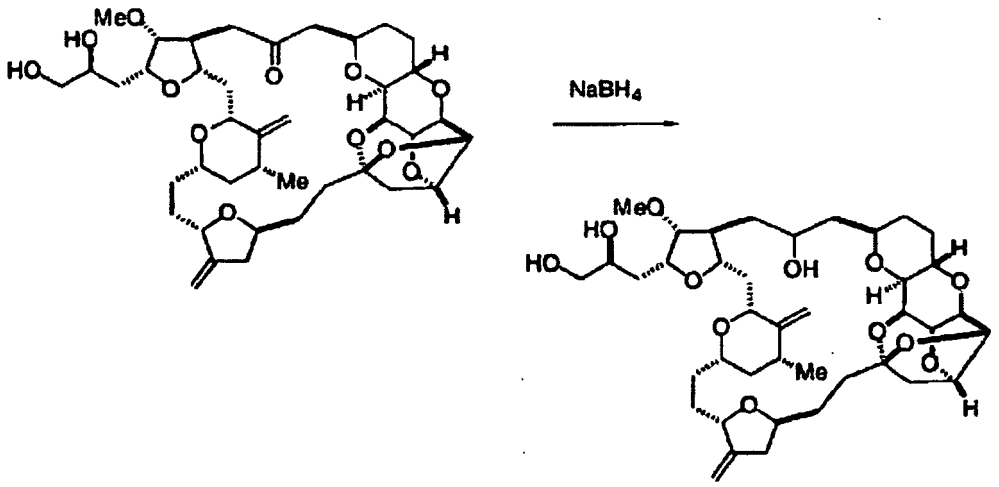


[0073] General. A mixture of **B1793** (1 mg, 1.37 micromol), Et_3N (10 microL, 72 micromol) and ArNCO (2 to 4 equiv.) in CH_2Cl_2 (0.2 mL) was stirred at rt for 4 h to overnight until the reaction was judged to be complete by TLC. The reaction mixture was diluted with saturated $NaHCO_3$ (3 mL), extracted with CH_2Cl_2 (3x) and EtOAc (2x), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH-CH_2Cl_2) to afford the products:

B1984. (1.0 mg, 86%) HRMS (FAB): calcd for $C_{47}H_{63}NO_{13}$ + Na 872.4197. Found: 872.4214.
B1990. (1.1 mg, 92%) HRMS (FAB): calcd for $C_{47}H_{62}ClNO_{13}$+ Na 906.3807. Found: 906.3826.
B1992. (1.0 mg, 83%) HRMS (FAB): calcd for $C_{48}H_{65}NO_{14}$ + Na 902.4303. Found: 902.4269.

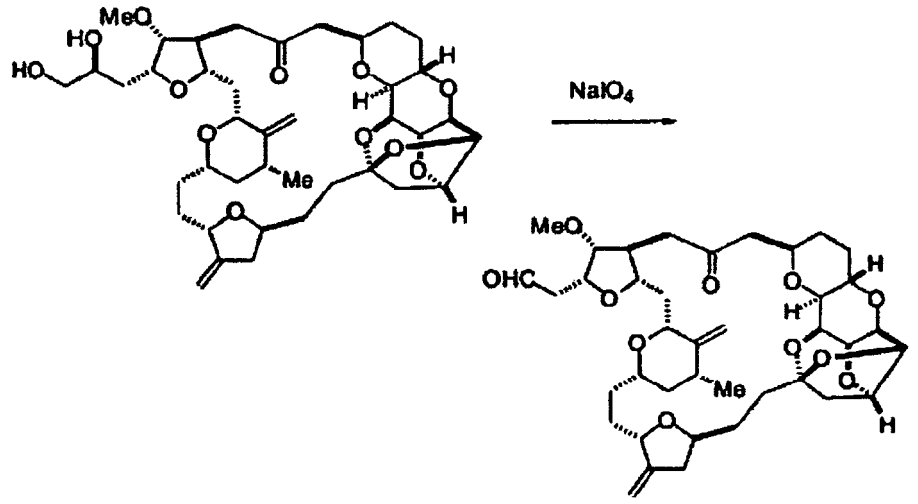


[0074] B2042 DEAD (0.4 M in ether, 50 μL, 19 μmol) was added to a solution of **B1793** (2.0 mg, 2.7 μmol), triphenylphosphine (5 mg, 19 μmol), 4-nitrobenzoic acid (3.2 mg, 19 μmol) and Et_2O (500 μL) at rt. After 22 h, the reaction mixture was loaded directly onto a preparative TLC plate and eluted with 60% EtOAc-hexanes to give the intermediate diester (3.0 mg). This material was taken up in MeOH (300 μL) and treated with K_2CO_3 (approximately 1 mg). After stirring at rt for 30 min, the reaction mixture was diluted with brine and extracted with CH_2Cl_2 (5x). The combined extracts were dried over Na_2SO_4, concentrated and purified by preparative TLC (5% MeOH-EtOAc) to afford **B2042** (1.2 mg, 60% for two steps). HRMS (FAB): calcd for $C_{40}H_{58}O_{12}$ + Na 753.3826. Found: 753.3810.



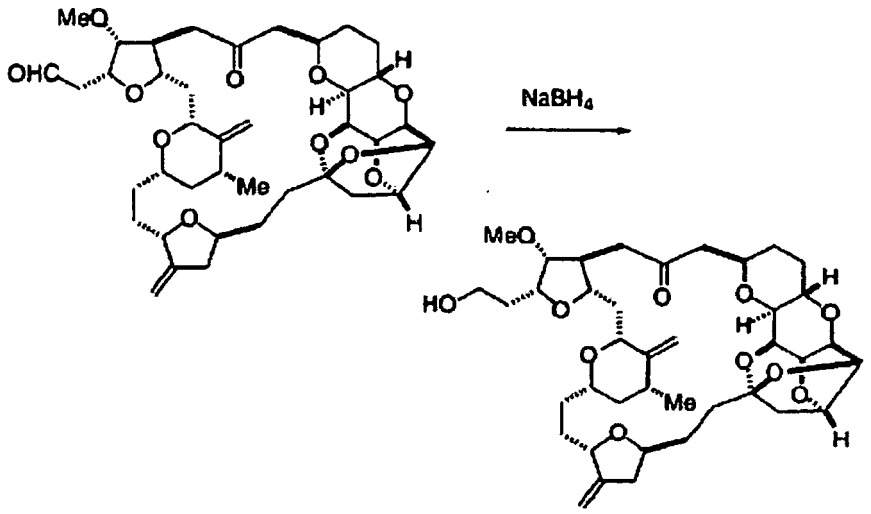
B1793 **B1896, B1897**

[0075] **B1896** and **B1897.** $NaBH_4$ (3 mg, 0.08 mmol) was added to a solution of **B1793** (2.30 mg, 3.15 μmol) in 1:1 CH_2Cl_2-MeOH (0.2 mL) at rt. Concentration of the reaction mixture and purification by preparative TLC (8% MeOH-EtOAc) provided **B1896** (0.80 mg, 35%) and **B1897** (2:1 mixture, 0.15 mg, 6.5%). HRMS (FAB) for **B1896:** calcd for $C_{40}H_{60}O_{12}$ + Na 755.3983. Found: 753.3969.



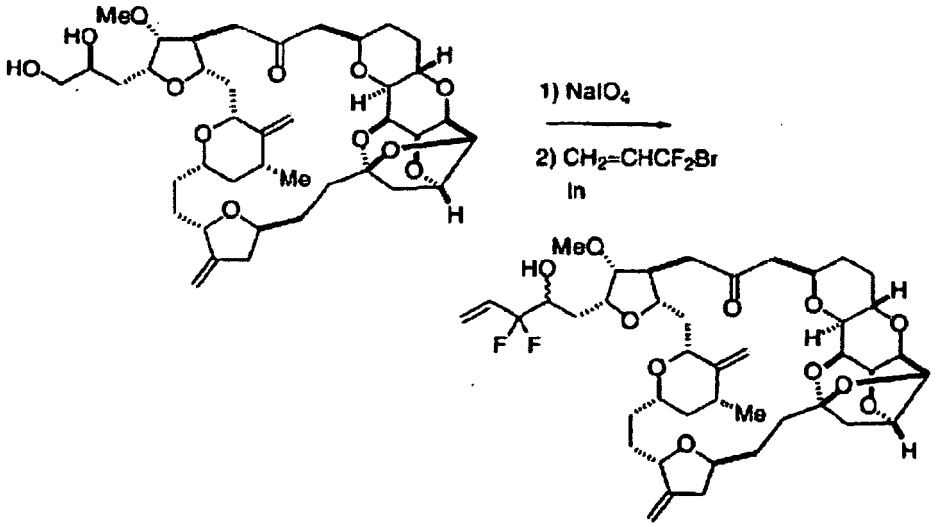
B1793 **B1918**

[0076] **B1918.** A mixture of **B1793** (2.0 mg, 2.74 μmol), $NaIO_4$ (35 mg, 0.16 mmol), MeOH (0.8 mL) and H_2O (0.2 mL) was stirred at rt for 40 min. The reaction mixture was diluted with H_2O (1 mL), extracted with CH_2Cl_2 (6x), dried over Na_2SO_4, concentrated and purified by column chromatography (5% MeOH-CH_2Cl_2) to give **B1918** (1.9 mg, 100%).



B1918 **B2037**

[0077] **B2037.** A 0.034 M solution of $NaBH_4$ (0.1 mL, 3.4 μmol) in EtOH was added portion-wise to a solution of **B1918** (1.9 mg, 2.72 μmol) in MeOH (0.8 mL) and CH_2Cl_2 (0.2 mL) at -78 °C to rt until the reaction was judged to be complete by TLC. The reaction was quenched with saturated aqueous NH_4Cl (2 mL) at -78 °C, warmed to rt, extracted with CH_2Cl_2 (6x), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH- CH_2Cl_2) to afford **B2037** (1.7 mg, 89%). HRMS (FAB): calcd for $C_{39}H_{56}O_{11}$ + Na 723.3720. Found: 723.3749.

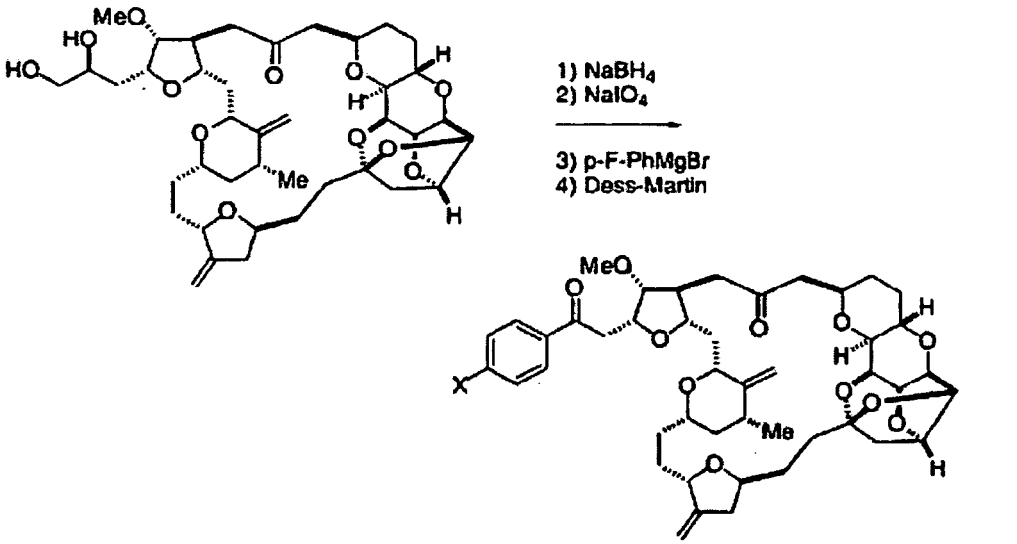


B1793 **B2035**

B2035

[0078] $NaIO_4$ (35 mg, 0.16 mmol) was added to a solution of **B1793** (1.7 mg, 0.0023 mmol), MeOH (800 μL) and H_2O (200 μL) and after 15 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (5x). The organic extracts were dried over Na_2SO_4, concentrated and the intermediate aldehyde was immediately dissolved in DMF (300 μL). 3-Bromo-3,3-difluoropropene (3μL, 0.023 mmol) and indium powder (3 mg, 0.23 mmol) were added and after 24 h additional 3-bromo-3,3-difluoropropene (1 μL, 0.008 mmol) was added. After 18 h, H_2O was added the mixture was extracted with EtOAc (3x). The combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide B2035 (0.74 mg, 41% for 2 steps) as a

mixture of isomers. HRMS (FAB): calcd for $C_{42}H_{58}F_2O_{11}$ + Na 799.3845. Found: 799.3883.



B1793

B2011: X = H

B2008: X = F

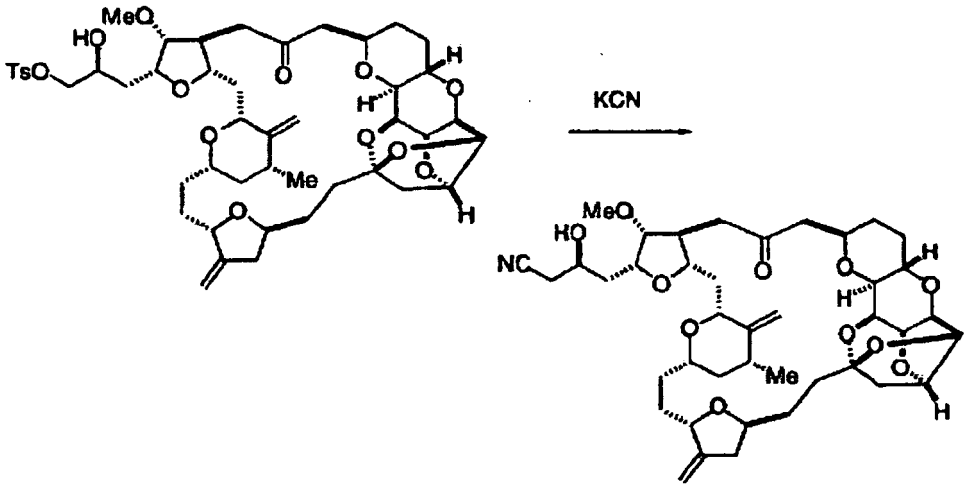
B2008, B2011

[0079] $NaBH_4$ (2 mg, 0.05 mmol) was added to a solution of **B1793** (2.2 mg, 0.003 mmol) in 1:1 CH_2Cl_2-MeOH (200 μL) at rt. After 15 min saturated aqueous NH_4Cl and H_2O were added, and the mixture was extracted with CH_2Cl_2 (6x) and EtOAc (2x). The combined organic extracts were dried over Na_2SO_4, concentrated and purified by column chromatography (10% MeOH-EtOAc) to provide an intermediate triol, which was dissolved in MeOH (800 μL) and H_2O (200μL). $NaIO_4$ (35 mg, 0.16 mmol) was added and after 20 min, the mixture was diluted with H_2O and extracted with CH_2Cl_2 (6x). The organic extracts were dried over Na_2SO_4, concentrated and the intermediate aldehyde was immediately dissolved in THF (500 μL).

4-Fluorophenylmagnesium bromide (2M in Et_2O, 12 μL, 0.024 mmol) was added and after 20 min the reaction was quenched with saturated aqueous NH_4Cl. The mixture was extracted with CH_2Cl_2 (6x) and the combined organic extracts were dried over Na_2SO_4 and concentrated. Purification by preparative TLC (EtOAc) provided the desired product as a mixture of 4 isomers (1.32 mg, 55% for 3 steps).

[0080] Dess-Martin periodinane (~3 mg, 0.007 mmol) was added to a solution of the above product (0.95 mg, 0.0012 mmol) in CH_2Cl_2 (300 μL) and the mixture was stirred at rt for 20 min. Additional Dess-Martin periodinane (~3 mg, 0.007 mmol) and CH_2Cl_2 (300 μL) were added and after another 40 min Et_2O, saturated aqueous $NaHCO_3$ (4 mL) and saturated aqueous $Na_2S_2O_3$ (1 mL) were added. The mixture was extracted with Et_2O (3x) and the combined extracts were washed with brine, dried over Na_2SO_4, concentrated and purified by column chromatography (20% EtOAc-hexanes) to provide **B2008** (0.58 mg, 61%). HRMS (FAB): calcd for $C_{45}H_{57}FO_{11}$ + Na 815.3783. Found: 815.3758.

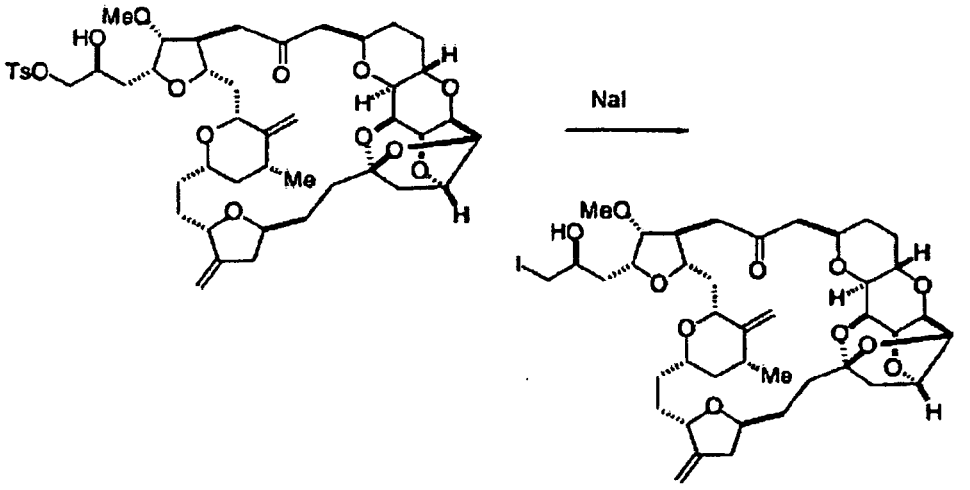
[0081] **B2011.** In an analogous manner, **B1793** (1.9 mg, 0.003 mmol) was converted to **B2011** (0.87 mg, 42% for 4 steps). HRMS (FAB): calcd for $C_{45}H_{58}O_{11}$ + Na 797.3877. Found: 797.3877.



B1920 **B2013**

B2013

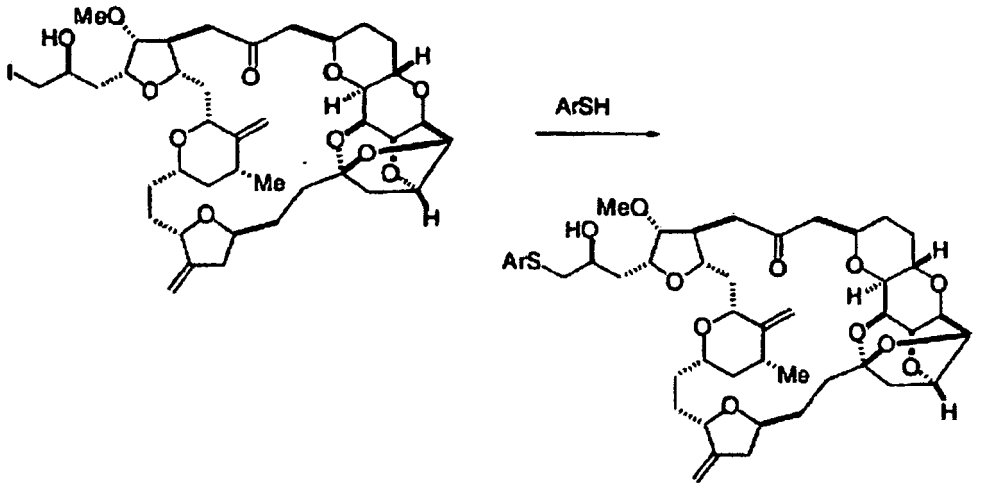
[0082] A solution of **B1920** (1.4 mg, 0.0016 mmol), KCN (1 mg, 0.016 mmol) and DMSO (500 μL) was heated at 60 °C for 8 h. After cooling to rt, H_2O was added and the mixture was extracted with EtOAc (3x). The combined organic extracts were washed successively with H_2O and brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to provide B2013 (0.78 mg, 67%). HRMS (FAB): calcd for $C_{41}H_{57}NO_{11}$ + Na 762.3829. Found: 762.3848.



B1920 **X1920**

X1920

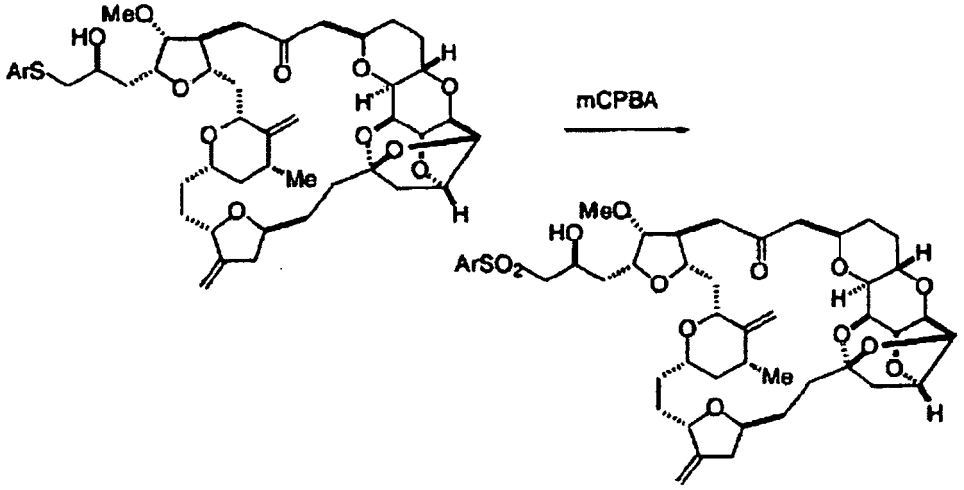
[0083] A mixture of **B1920** (1.3 mg, 1.47 μmol), NaI (30 mg, excess) and acetone (1 mL) was stirred at 60 °C for 3.5 h. After cooling to rt, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (3 mL), extracted with CH_2Cl_2 (5x) and EtOAc, dried over Na_2SO_4 and purified by column chromatography (50% EtOAc-CH_2Cl_2 to 80% EtOAc-hexanes) to give the iodide **X1920** (1.3 mg, 100%).



X1920

B1998: Ar = *p*-Cl-Ph
B2010: Ar = *p*-MeO-Ph
B2019: Ar = 2-imidazole

[0084] General. A mixture of iodide **X1920** (1.0 equiv.), iPr_2EtN (11 to 22 equiv.), ArSH (9 to 46 equiv.) and DMF (0.3 mL) was stirred at rt until the reaction was judged to be complete by TLC (typically 24 to 48 h). The reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or 5% MeOH-CH_2Cl_2) to afford the sulfide products:
B1998. (1.3 mg gave 1.1 mg, 85%) HRMS (FAB): calcd for $C_{46}H_{61}ClO_{11}S$ + Na 897.3521. Found: 897.3533.
B2010. (1.1 mg gave 0.7 mg, 59%). HRMS (FAB): calcd for $C_{47}H_{64}O_{12}S$ + Na 875.4016. Found: 875.4057.
B2019. (1.1 mg gave 0.7 mg, 61%) MS (FAB): M + Na

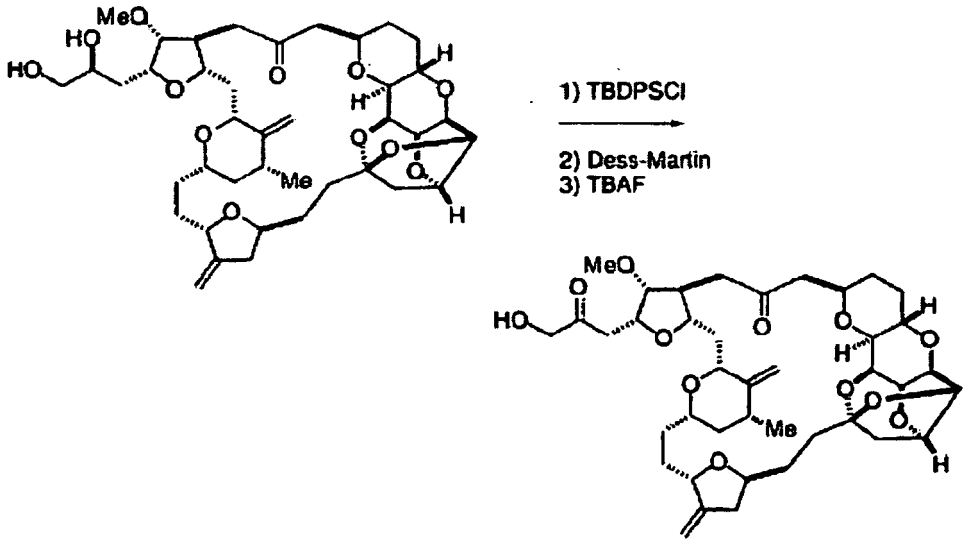


B1998: Ar = *p*-Cl-Ph
B2010: Ar = *p*-MeO-Ph

B2016: Ar = *p*-Cl-Ph
B2030: Ar = *p*-MeO-Ph

[0085] General. A 0.01 M solution of mCPBA (1.2 equiv.) in CH_2Cl_2 was added to a solution of a sulfide (1.0 equiv.) in CH_2Cl_2 (0.5 mL) at 0 °C for 30 min. The reaction mixture was diluted with saturated $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 and EtOAc, dried over Na_2SO_4 and purified by preparative TLC (80% EtOAc-hexanes or EtOAc) to afford the products:

B2016. (0.9 mg gave 0.7 mg, 74%)
B2030. (1.0 mg gave 0.6 mg, 61%) HRMS (FAB): calcd for $C_{47}H_{64}O_{14}S$ + Na 907.3914. Found: 907.3950.



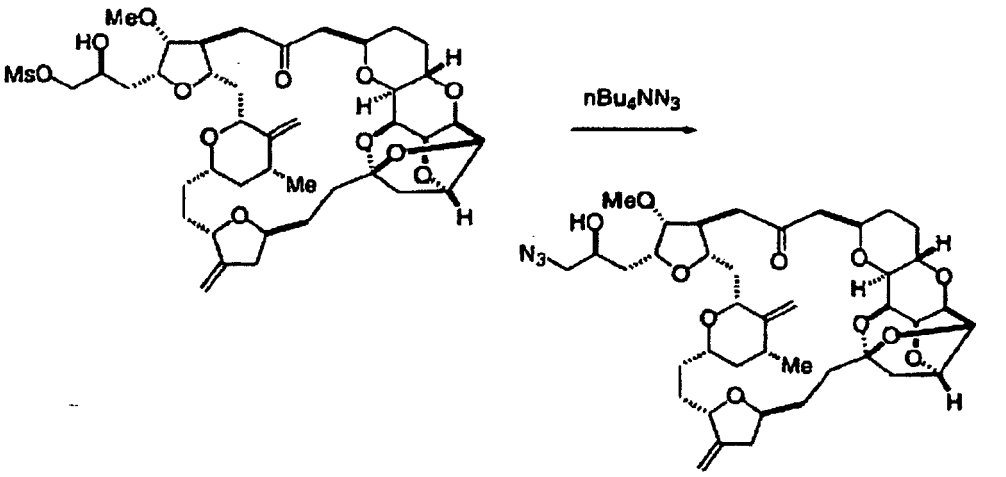
B1793 **B1934**

[0086] **B1934.** TBDPSCl (3.0 μL, 12 μmol) was added to a solution of **B1793** (1.3 mg, 1.78 μmol), imidazole (10 mg, 166 μmol) and DMF (0.10 mL) at rt. After stirring for 1 h, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL), extracted with CH_2Cl_2 (3x) and EtOAc (2x), dried over Na_2SO_4 and purified by preparative TLC (5% MeOH-CH_2Cl_2) to give the intermediate silyl ether (1.3 mg, 77%).

[0087] This material was dissolved in CH_2Cl_2 (0.5 mL) and treated with Dess-Martin periodinane (10 mg, 24 μmol) for 1.5 h at rt, diluted with Et_2O and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to afford the diketone intermediate (1.0 mg, 77%), which was dissolved in THF (0.5 mL) and treated with 0.02 M TBAF containing 0.01 M imidazole hydrochloride (THF solution, 75 μL, 1.5 μmol) at rt for 15 min. The reaction mixture was eluted through a SiO_2 column (50% EtOAc-hexanes to 5% MeOH-CH_2Cl_2) and the desired product was further purified by preparative TLC (5% MeOH-CH_2Cl_2) to afford **B1934** (0.75 mg, 100%). HRMS (FAB): calcd for $C_{40}H_{56}O_{12}$ + Na 751.3669. Found: 751.3690.

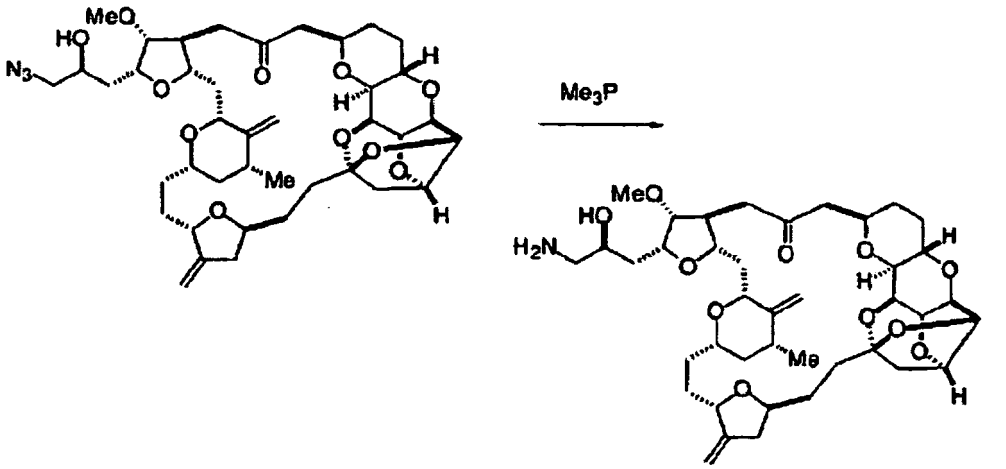
Synthesis of B1939:

[0088]



B2294 **B1922**

[0089] **B1922** Tetra-n-butylammonium azide (0.2 M in DMF, 0.5 mL, 0.10 mmol) was added to a solution of mesylate **B2294** (21.4 mg, 0.026 mmol) in DMF (2 mL) at rt. After stirring at 83 °C for 3.5 h, the reaction mixture was cooled to rt, diluted with toluene, concentrated and purified by preparative TLC (80% ethyl acetate-hexanes) to furnish **B1922** (18 mg, 92%).

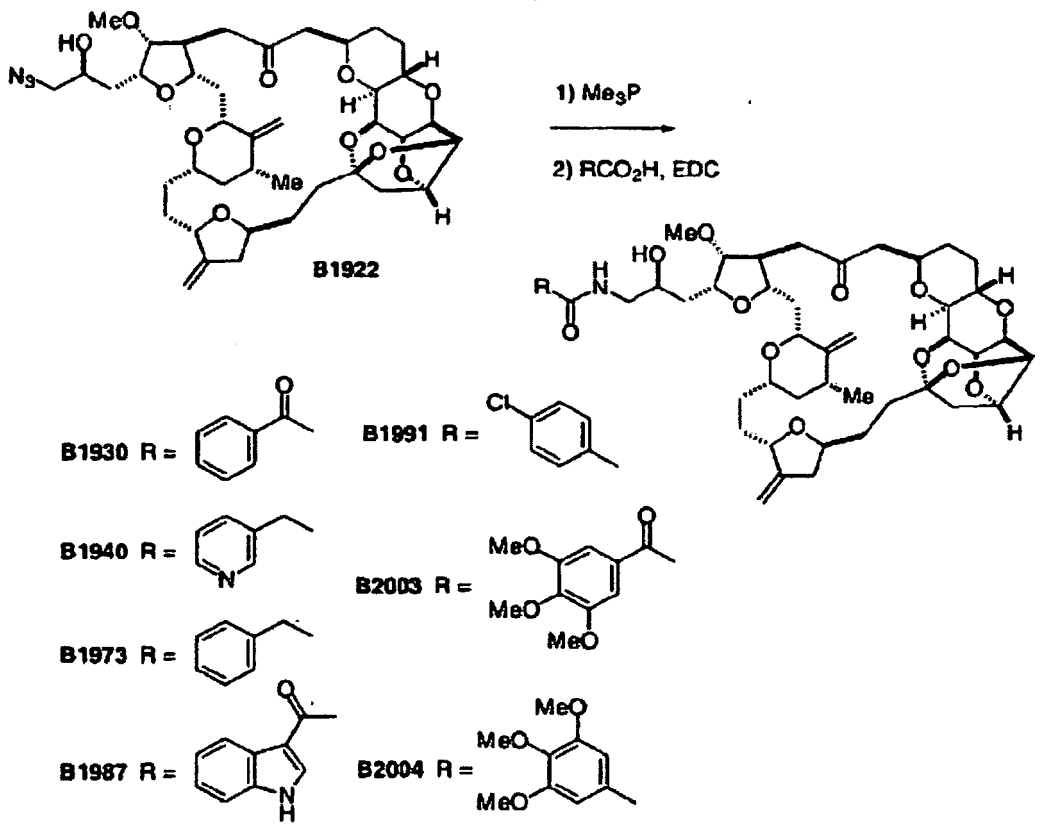


B1922 **B1939**

[0090] **B1939** Me_3P (1 M in THF) and H_2O (0.8 mL) were sequentially added to a solution of azide **B1922** (24.6 mg, 0.032 mmol) in THF (3.2 mL) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated and purified by flash chromatography [step gradient, 10% MeOH-EtOAc followed by MeOH-EtOAc-30% aqueous NH_4OH (9:86:5)] to provide the desired primary amine (23.3 mg), which by ^1H-NMR contained ~1% trimethylphosphine oxide. Lyophilization from benzene and standing under high vacuum for 2 d furnished **B1939** (20.3 mg, 87%).

Synthesis of Representative B1939 Analogs:

[0091] *B1930, B1940, B1973, B1987, B1988, B1991, B2003, B2004*



[0092] **B1930** Me_3P (1M in THF, 13 μL, 0.013 mmol) was added to a solution of **B1922** (1.6 mg, 2.1 μmol), THF (400 μL) and H_2O (100 μL) at rt. The mixture was stirred for 22 h, diluted with toluene, concentrated, and azeotropically dried with toluene (2x) to give the crude amine which was used directly in the next step.

[0093] EDC (0.06 M in CH_2Cl_2, 100 μL, 11 μmol) was added to a solution of the crude amine, benzoylformic acid (0.8 mg, 5.3 μmol) and CH_2Cl_2 (200 μL) at rt. After 30 min, the reaction was quenched with a 1:4 mixture of saturated aqueous $NaHCO_3$-brine and extracted with CH_2Cl_2 (5x). The combined extracts were dried over Na_2SO_4 and concentrated and purified by preparative TLC (EtOAc) to afford **B1930** (1.5 mg, 83% for two steps). HRMS (FAB): calcd for $C_{48}H_{63}NO_{13}$ + Na 884.4197. Found: 884.4166.

[0094] **B1940** Using the procedure described above for **B1930, B1922** was reduced, coupled with 3-pyridylacetic acid hydrochloride and purified by preparative TLC [(MeOH-EtOAc-30% aqueous NH_40H (9:86:5)] to afford **B1940** (0.8 mg, 67 % for two steps). HRMS (FAB): calcd for $C_{47}H_{64}N_2O_{12}$ + Na 871.4357. Found: 871.4362.

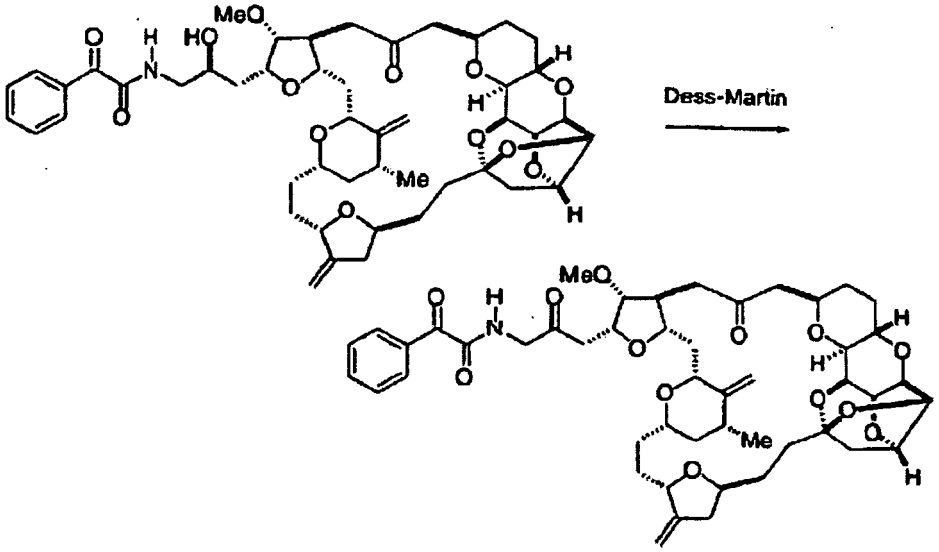
[0095] **B1973** Using the procedure described above, **B1922** (0.9 mg, 1.2 μmol) was reduced, coupled with phenylacetic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford **B1973** (0.44 mg, 44 % for two steps). HRMS (FAB): calcd for $C_{48}H_{65}NO_{12}$ + Na 870.4404. Found: 870.4447.

[0096] **B1987** Using the procedure described above, **B1922** (0.9 mg, 1.2 μmol) was reduced, coupled with 3-indoleglyoxylic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford B1987 (0.8 mg, 75 % for two steps). HRMS (FAB): calcd for $C_{50}H_{64}N_2O_{13}$ + Na 923.4306. Found: 923.4338.

[0097] **B1991** Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 4-chlorobenzoic acid and purified by preparative TLC (3% MeOH-EtOAc) to afford **B1991** (0.8 mg, 70 % for two steps). HRMS (FAB): calcd for $C_{47}H_{62}ClNO_{12}$ + Na 890.3858. Found: 890.3843.

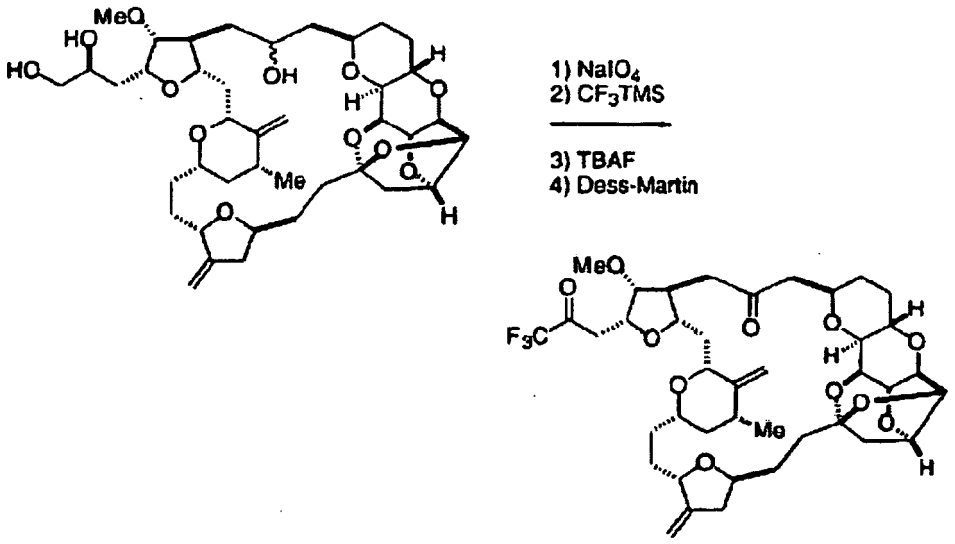
[0098] **B2003** Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 3,4,5-trimethoxybenzoylformic acid and purified by preparative TLC (EtOAc) to afford **B2003** (0.7 mg, 56 % for two steps). HRMS (FAB): calcd for $C_{51}H_{69}NO_{16}$ + Na 974.4514. Found: 974.4525.

[0099] **B2004** Using the procedure described above, **B1922** (1.0 mg, 1.3 μmol) was reduced, coupled with 3,4,5-trimethoxybenzoic acid and purified by preparative TLC (5% MeOH-EtOAc) to afford B2004 (0.7 mg, 58 % for two steps). HRMS (FAB): calcd for $C_{50}H_{69}NO_{15}$ + Na 946.4565. Found: 946.4599.



B1930 **B1988**

[0100] **B1988** Dess-Martin periodinane (1 mg, 2.3 μmol) was added to a solution of **B1930** (0.80 mg, 0.93 μmol) in CH_2Cl_2 (500 μL) at rt. After 1 h, the reaction was diluted with Et_2O and filtered through Celite. The filtrate was washed sequentially with a 1:9 mixture of saturated aqueous $NaHCO_3$-$Na_2S_2O_3$ and brine, dried over Na_2SO_4, concentrated and purified by preparative TLC (80% EtOAc-hexanes) to afford **B1988** (0.45 mg, 56%). HRMS (FAB): calcd for $C_{48}H_{61}NO_{13}$ + Na 882.4041. Found: 884.4012.



B1896, B1897 **B1942**

[0101] **B1942.** A mixture of **B1896/B1897** (2 mg, 2.73 μmol), $NaIO_4$ (35 mg, 0.16 mmol), MeOH (0.8 mL) and H_2O (0.2 mL) was stirred at rt for 30 min. The reaction mixture was then diluted with H_2O (3 mL) and extracted with CH_2Cl_2 (6x) and EtOAc (2x). The combined organic phases were dried over Na_2SO_4 and purified by column chromatography (5% MeOH- CH_2Cl_2) to give the desired aldehyde.

[0102] This material was dissolved in THF (0.1 mL), cooled to at 0 °C and treated with 0.5 M CF_3TMS in THF (30 μL,

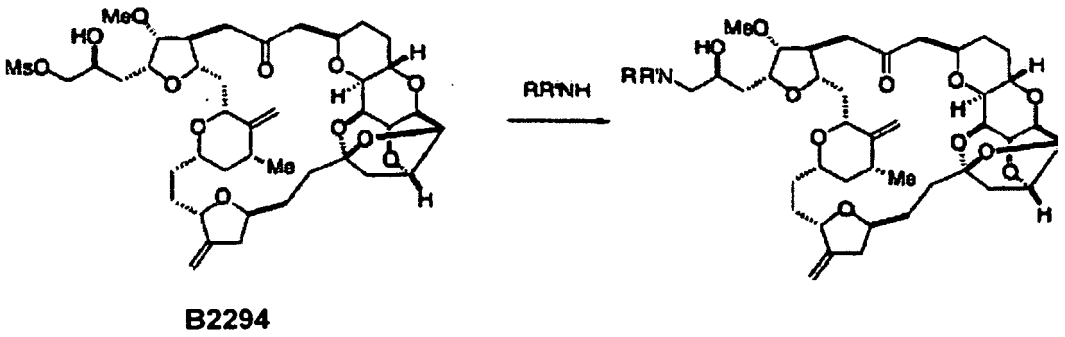
15 mmol) followed by 0.05 M TBAF in THF (5 mL, 0.025 mmol). After stirring for 30 min, the reaction mixture was diluted with saturated aqueous $NaHCO_3$ (2 mL) and H_2O (1 mL), extracted with EtOAc (6x), dried over Na_2SO_4, filtered and concentrated to give the crude bis-TMS ether.

[0103] This material was dissolved in THF (0.5 mL) and treated with 1 M TBAF in THF containing 0.5 M imidazole hydrochloride (8 μL, 8 μmol) at rt for 30 min. The reaction mixture was eluted through a SiO_2 column (50% EtOAc-hexanes to EtOAc) to afford the diol intermediate.

[0104] A mixture of this product and Dess-Martin periodinane (10 mg, 24 mmol) in CH_2Cl_2 (0.5 mL) was stirred at rt for 1 h, diluted with Et_2O (5 mL) and filtered through Celite. The filtrate was concentrated and purified by preparative TLC (50% EtOAc-hexanes) to furnish B1942 (1.5 mg, 72% for 5 steps). HRMS (FAB): calcd for $C_{40}H_{53}F_3O_{11}$ + H 767.3516. Found: 767.3542

Synthesis of B2320 and Related Analogs

[0105]

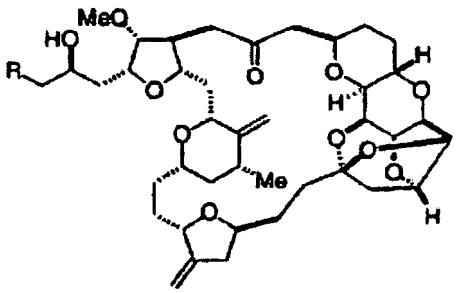


B2294

[0106] These compounds are made by treating B2294 with an appropriate amine in a solvent such as methanol for a period of a few hours to several days. Progress of the reaction may be monitored by thin layer chromatography. A standard work-up procedure, well known to those of skill in the art, provides the desired compounds. The procedure below is to prepare ER803868; however this procedure is general and can be used to prepare any desired analog.

Synthesis of ER803868

[0107] To a solution of B2294, 1.2 mg, in methanol, 0.5 mL, was added morpholine, 0.012 mL. The mixture was stirred for 10 days with additional morpholine, 0.012 mL, being added on days 1,2,3, 4 and 8. The mixture was then chromatographed to give 1.4 mg of the desired compound.



B2320	R = N,N-dimethylamino
B2330	R = N-isopropylamino
B2336	R = N-methylamino
B2339	R = N-t-butylamino

(continued)

B2417	R = N-2-hydroxyethylamino
B2418	R = N-piperazinyl
B2489	R = N,N-bis-(2-hydroxyethyl)amino
B2490	R = N-1,3-dihydroxy-2-propylamino
B2491	R = N-benzylamino
ER803834	R = N-piperidinyl
ER803835	R = N-pyrrolidinyl
ER803836	R = N-3-(R)-hydroxypyrrolidinyl
ER803843	R = N-homopiperidinyl
ER803845	R = N-para-methoxybenzylamino
ER803846	R = N-phenethylamino
ER803851	R = N-2-(S-hydroxymethyl)pyrrolidinyl
ER803852	R = N-2-(R-hydroxymethyl)pyrrolidinyl
ER803868	R = N-morpholinyl
ER803869	R = N-ethylamino
ER803870	R = N-imidazoyl
ER803883	R = N,N-diethylamino
ER803884	R = N-para-chlorobenzylamino

D. Pharmacological Activity

[0108] Many of the individually disclosed drugs were tested for *in vitro* and *in vivo* activity (see Table 1, below). Screening methods included a standard *in vitro* cell growth inhibition assay using DLD-1 human colon cancer cells (ATCC accession number CCL 221) in a 96-well microtiter plate format (Finlay, G.J. et al Analytical Biochemistry 139: 272-277, 1984), a U937 (ATCC accession number CRL 1593) mitotic block reversibility assay (described below), and in some cases, a LOX human melanoma tumor xenograft *in vivo* growth inhibition assay (see Table 1). Chemical stability to esterase degradation was also examined.

U937 Mitotic Block Reversibility Assay

[0109] U937 human histiocytic lymphoma cells were added to 75 cm^2 tissue culture flasks as 2.5 x 10^6 cells in 22.5 mL of RPMI Medium 1640 containing 10% Fetal Bovine Serum. Cells were allowed to adapt to the culture during 36 h of incubation at 37 °C in a humidified atmosphere containing 5% CO_2. Each test drug was then added to a flask as 2.5 mL of 10x final concentration. Final concentrations achieved were 0.1-1000 nM, in half log-increments, for a total of 10 concentration steps including a drug-free control flask which received 2.5 mL of media. Cells were incubated with drug for 12 h pretreatment period at 37 °C in a humidified atmosphere containing 5% CO_2.

[0110] The contents were removed from each flask and centrifuged at 300 x g for 10 min at room temperature, after which drug-containing media was removed from cell pellet. Cells were resuspended in 25 mL of warm drug-free media and centrifuged at 300 x g for 10 min at room temperature. After removing media from cell pellet, cells were resuspended in 35 mL of warm drug-free media, transferred to fresh flasks, and a 10 mL sample of cells immediately removed from each flask, immediately processed as described below and stored for later cell cycle analysis (0 hours of drug washout).

[0111] Incubation of the remaining 25 mL of cells continued in drug-free media for another 10 h. A 10 mL sample of cells was removed from each flask, immediately processed and stored for later cell cycle analysis (10 hours of drug washout) and 10 mL fresh replacement media was added to each incubation flask. Incubation of cells in drug-free media continued for 5 days. At day two, 20 mL of media and cells was removed from each flask and replaced with 20 mL fresh media. Viability of cells was quantified after 5 days by trypan blue exclusion techniques using hemacytometer counting.

[0112] Cells were processed for cell cycle analysis using modifications of the method published in Becton Dickinson Immunocytometry Systems source book section 1.11 (Preparation of Alcohol-Fixed Whole Cells From Suspensions For DNA Analysis). Briefly, each 10 mL sample of cells removed from the flasks at 0 and 10 hours of drug washout was separately centrifuged at 300 x g for 10 min. After removing the media from the cell pellet, cells were resuspended in 3 mL cold saline. Seven milliliters cold 100 % ethanol was slowly added with vigorous vortexing. Ethanol treated cell samples from 0 hour and 10 hour periods of compound washout were stored overnight at 4 ° C. Ethanol treated cells were centrifuged 300 x g for 10 min, ethanol removed and cells then washed in 10 mL Phosphate Buffered Saline (PBS). Cells were resuspended in 0.5 mL of 0.2 mg/mL Ribonuclease A (Sigma No. R-5503) in PBS and incubated in 37 ° C

water bath for 30 min.

[0113] Cells were transferred to appropriate flow cytometry tubes and 0.5 mL of 10 mg/mL propidium iodide (PI) (Sigma No. P4170) in PBS was added to each tube. Cells were incubated with PI at room temperature in the dark for at least 15 min prior to analysis with a flow cytometer (Becton Dickinson FACScan flow cytometer or equivalent). Cells should be analyzed within an hour and kept in the dark at 4 ° C until ready. Cell cycle analysis was performed on 0 hour and 10 hour cells using flow cytometric measurement of the intensity of cellular fluorescence. The intensity of propidium iodide fluorescence for each cell was measured on a linear amplification scale with doublet events ignored using doublet discrimination. The results obtained from analyzing 15,000 cells were presented as a histogram with increasing fluorescence intensity on the x-axis and the number of cells at a particular intensity level on the y-axis.

[0114] The intensity of PI staining is dependent on the amount of DNA in the cell so it is possible to identify cells in various phases of the cell cycle, such as cells that have not yet synthesized DNA since the last mitosis (G_1 phase), cells that are in intermediate stages of DNA synthesis (S phase), and cells that have doubled their complement of DNA and are ready to divide (G_2 phase). Cells that are blocked in the mitosis phase of the cell cycle also have double the amount of DNA compared to G_1 phase cells. If all cells are blocked in mitosis there are no G_1 phase cells, but if the block is removed when compound is removed, cells complete mitosis and reappear in the G_1 phase. The number of cells so reappearing in the G_1 or Sphase is thus a measure of the number of cells which have recently completed mitosis. For each sample at 0 and 10 hours after compound removal, the percentage of cells completing mitosis was quantified (as the number of cells reappearing in the G_1 phase) and plotted as a function of the initial concentration of compound used during the 12 hour pretreatment period. The percentage of cells still viable 5 days after drug washout was superimposed on the same graph. A ratio can be determined between the compound concentration required to completely block all cells in mitosis at 0 hour and the concentration required to maintain the block 10 hours after compound removal. This was taken as a measure of a compound's reversibility, with ratios close to or equal to one indicating likely potent *in vivo* anti-tumor compounds (see Table 1, columns 4-6).

Table 1

In Vitro Inhibition and Reversibility Data					
	DLD-1*		Complete Mitotic Block		Reversibility Ratio
compound	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	
B1793	0.93	0.04	3	44	14.7
B1918	1.27	0.12			
B1920	2.00	0.15			
B1921	24.00	1.15			
B1922	0.53	0.01	3	30	10.0
B1930	0.87	0.03			
B1934	1.05	0.21	3	30	10.0
B1939	19.34	2.36	12	12	1.0
B1940	5.43	0.62			
B1942	0.60	0.03	3	30	10.0
B1973	1.15	0.24			
B1984	1.01	0.15			
B1987	1.82	0.21			
B1988	2.67	1.02			
B1990	1.30	0.06			
B1991	0.69	0.03			
B1992	0.86	0.07			
B1998	1.23	0.13			
B2003	1.21	0.12			

(continued)

In Vitro Inhibition and Reversibility Data					
	DLD-1*		Complete Mitotic Block		Reversibility Ratio
compound	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	
B2004	0.63	0.04			
B2008	2.63	0.63			
B2010	0.71	0.12			
B2011	1.81	0.52			
B2013	0.49	0.07	2	30	15.0
B2014	0.87	0.09			
B2015	2.78	0.23			
B2016	0.66	0.06			
B2019	0.82	0.07			
B2035	0.76	0.09			
B2037	0.66	0.11			
B2042	1.93	0.11	3	100	33.3
B2294	0.80	0.01			
B2320	1.20	0.17	1	10	10.0
B2330	4.40	0.42	7	7	1.0
B2336	3.33	0.09	10	10	1.0
B2339	4.30	0.21	3	3	1.0
B2417	12.67	0.33	10	10	1.0
B2418	3.63	0.17	10	10	1.0
B2489	14.67	2.03	10	10	1.0
B2490	35.67	3.33	100	100	1.0
B2491	0.92	0.14	2	10	6.7
ER803834	0.47	0.08	1	10	10.0
ER803835	15.33	0.33	10	10	1.0
ER803836	1.97	0.12	3	10	3.3
ER803843	0.49	0.05	1	10	10.0
ER803845	1.50	0.20	3	10	3.3
ER803846	1.16	0.10	1	10	10.0
ER803851	3.33	0.26	3	3	1.0
ER803852	3.03	0.50	3	10	3.3
ER803868	0.43	0.03	3	10	3.3
ER803869	4.13	0.64	3	3	1.0
ER803870	1.27	0.12	3	30	10.0
ER803883	1.02	0.04	1	10	10.0

(continued)

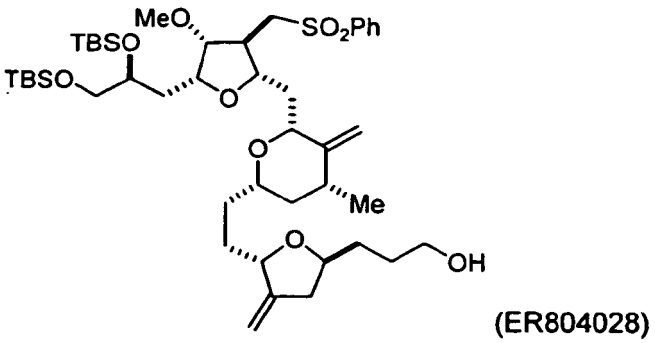
In Vitro Inhibition and Reversibility Data					
	DLD-1*		Complete Mitotic Block		Reversibility Ratio
compound	mean IC50, nm	SE	0 hour, nM**	10 hour, nM‡	
ER803884	0.59	0.03	1	10	10.0
* = in vitro cell growth inhibition ** = before washout = after washout					

E. Use

[0115] The disclosed analog compounds have pharmacological activity, including anti-tumor and anti-mitotic activity as demonstrated in section D above. Examples of tumors include melanoma, fibrosarcoma, monocytic leukemia, colon carcinoma, ovarian carcinoma, breast carcinoma, osteosarcoma, prostate carcinoma, lung carcinoma and ras-transformed fibroblasts.

Claims

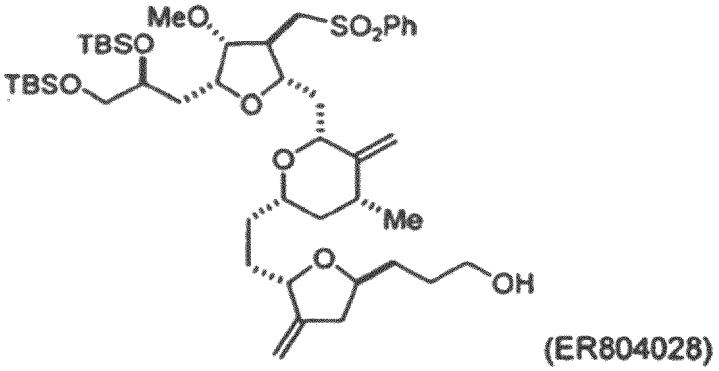
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(ER804028)

Patentansprüche

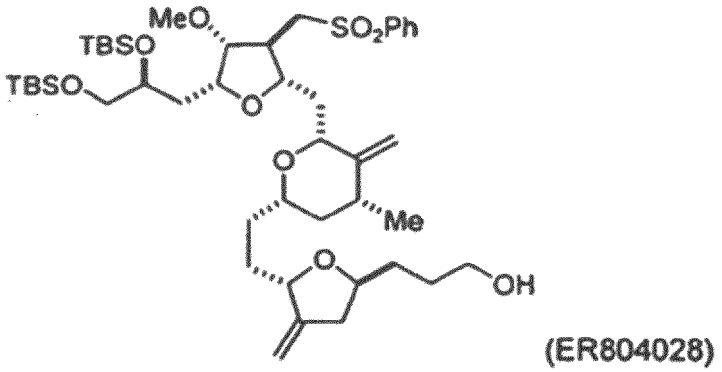
1. Verbindung mit der Formel:



(ER804028)

Revendications

1. Composé présentant la formule:



(ER804028)

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- EP 0572109 A **[0003]**
- WO 9317690 A **[0004]**

Non-patent literature cited in the description

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- **B. Aicher, T. D. ; Buszek, K. R. ; Fang, F. G. ; Forsyth, C. J. ; Jung, S. H. ; Kishi, Y. ; Matelich, M. C. ; Scola, P. M. ; Spero, D. M. ; Yoon, S. K.** *J. Am. Chem. Soc.,* 1992, vol. 114, 3162-4 **[0025]**
- **Stamos, D. P. ; Kishi, Y.** *Tetrahedron Lett.,* 1996, vol. 37, 8643-8646 **[0025]**
- **Finlay, G.J. et al.** *Analytical Biochemistry,* 1984, vol. 139, 272-277 **[0108]**